University of Helsinki
Faculty of Medicine

LINGONBERRY JUICE, BLOOD PRESSURE, VASCULAR FUNCTION AND INFLAMMATORY MARKERS IN EXPERIMENTAL HYPERTENSION

Anne Kivimäki

Doctoral Programme in Biomedicine (DPBM)
Doctoral School in Health Sciences (DSHealth)

Pharmacology
Faculty of Medicine
University of Helsinki

DOCTORAL DISSERTATION

To be presented for public discussion with the permission of the Faculty of Medicine of University of Helsinki, in Lecture Hall 3,
Biomedicum Helsinki, Haartmaninkatu 8, on 13th December 2019, at 12 noon.

HELSINKI 2019

SUPERVISORS

Professor (*emer.*) Heikki Vapaatalo, MD, PhD
Medical Faculty, Pharmacology
University of Helsinki

Professor Riitta Korpela, PhD
Medical Faculty, Pharmacology
University of Helsinki

REVIEWERS

Pertti Arvola, MD, PhD, Docent
Tampere University Hospital, Infectious Diseases Unit
University of Tampere

Riitta Törrönen, PhD, Docent
Institute of Public Health and Clinical Nutrition
University of Eastern Finland

OPPONENT

Professor Seppo Salminen, PhD
Faculty of Medicine, Functional Foods Forum
University of Turku

ISBN 978-951-51-5630-3 (print)
ISBN 978-951-51-5631-0 (PDF)
http://ethesis.helsinki.fi
Unigrafia Oy 2019

The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertions.

It´s all about balance.

2 TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS 6

MAIN ABBREVIATIONS 7

ABSTRACT 9

1 INTRODUCTION 10

2 REVIEW OF THE LITERATURE 11

2.1 **Regulation of blood pressure** 11

2.1.1 Sympathetic nervous system 12

2.1.2 Renin-angiotensin system 13

2.1.3 Kallikrein-kinin system 16

2.1.4 Kidneys as a regulator of blood pressure 17

2.2 **Vascular function** 20

2.2.1 Arterial structure 20

2.2.2 Vasoactive factors released from endothelium 22

2.3 **Vascular inflammation and oxidative stress** 26

2.3.1 Inflammatory response and NF-κB 26

2.3.2 Prostanoids and COX enzymes 27

2.3.3 CRP and alkaline phosphatase as diagnostic tools 29

2.4 **Hypertension and vascular dysfunction – pharmacological and dietary treatments** 31

2.4.1 Pharmacological treatment of hypertension 33

2.4.2 Non-pharmacological treatment of hypertension 33

2.5 **Berries in the prevention of hypertension and vascular diseases** 36

2.5.1 Epidemiological studies on berry consumption and health 36

2.5.2 Clinical studies 37

2.5.3 Effects of lingonberry on cardiovascular health 40

2.6 **Polyphenols in berries** 42

2.6.1 Flavonoids 42

2.6.2 Lingonberry and its polyphenols 45

2.7 **Bioavailability and safety aspects of polyphenols** 47

2.7.1 Bioavailability and food matrix interactions 47
2.7.2 Safety issues 48
3 AIMS OF THE STUDY 50
4 MATERIALS AND METHODS 51
4.1 **Study models and designs** 51
4.1.1 Animal models (Study I-IV) 51
4.1.2 Berry juices (Study I-IV) 52
4.2 **Methods** 53
4.2.1 Blood pressure measurement (Study I, III, IV) 53
4.2.2 Vascular reactivity studies (Study I, III, IV) 53
4.2.3 Biochemistry and clinical chemistry (Studies II-IV) 54
4.2.4 Immunohistochemistry (additional data) 56
4.2.5 Molecular docking (additional data) 56
4.2.6 Data analyses 57
5 RESULTS 58
5.1 **Weight gain, food and fluid consumption (Studies I-IV)** 58
5.2 **Polyphenol intake (Studies I-IV)** 58
5.3 **Blood pressure (Studies I, III and IV)** 59
5.4 **Vascular reactivity (Studies I, III and IV)** 60
5.5 **Renin-angiotensin system and nitric oxide** (Studies II-IV) 62
5.6 Inflammation and COX2 (Studies II, III and IV) 63
5.6.1 Clinical chemistry 63
5.6.2 Inflammatory variables and effects of salt-loading 64
5.6.3 Gene expressions 65
5.6.4 COX2 and the kidneys (additional data) 65
5.6.5 Molecular docking, COX2 66
6 DISCUSSION 68
7 SUMMARY AND CONCLUSIONS 75
8 ACKNOWLEDGEMENTS 76
9 REFERENCES 78

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on following publications (Studies I-IV) and some additional data. Original publications are printed with kind permission of the copyright owners.

I **Kivimäki AS**, Ehlers PI, Turpeinen AM, Korpela R, Vapaatalo H. Lingonberry juice improves endothelium-dependent vasodilatation of mesenteric arteries in spontaneously hypertensive rats in a long-term intervention. Journal of Functional Foods. 2011;3:267-274.

II **Kivimäki AS**, Ehlers PI, Siltari A, Turpeinen AM, Vapaatalo H, Korpela R. Lingonberry, cranberry and blackcurrant juices affect mRNA expressions of inflammatory and atherothrombotic markers of SHR in a long-term treatment. Journal of Functional Foods. 2012;4:496-503.

III **Kivimäki AS**, Siltari A, Ehlers PI, Korpela R, Vapaatalo H. Lingonberry juice lowers blood pressure of spontaneously hypertensive rats (SHR). Journal of Functional Foods. 2013;5:1432-1440.

IV **Kivimäki AS**, Siltari A, Ehlers PI, Korpela R, Vapaatalo H. Lingonberry juice negates the effects of a high salt diet on vascular function and low-grade inflammation. Journal of Functional Foods. 2014;7:238-245.

MAIN ABBREVIATIONS

ACE1,2	angiotensin-converting enzyme 1, 2
Ach	acetylcholine
ADMA	asymmetric dimethylarginine
Alb	albumin
ALP	alkaline phosphatase
Ang 1-7	Angiotensin 1-7
Ang I, II	Angiotensin I, II
AT1R, -2R	angiotensin II receptor type 1,2
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
COX1, 2	cyclooxygenase 1, 2
CRP	C-reactive protein
DBP	diastolic blood pressure
EDHF	endothelium-derived hyperpolarizing factor
EDRF	endothelium-derived relaxing factor
EFSA	European Food Safety Authority
eNOS	endothelial nitric oxide synthase
ET1	endothelin 1
HDL	high-density lipoprotein
H_2O_2	hydrogen peroxide
HS	high-salt
ICAM	intracellular adhesion molecule
iNOS	inducible nitric oxide synthase
KKS	kallikrein-kinin system
LDL	low-density lipoprotein
MAP	mean arterial pressure
MasR	Mas-receptor
MCP1	monocyte chemoattractant protein 1
NF-KB	nuclear factor kappa B

nNOS	neuronal nitric oxide synthase
NO	nitric oxide
Nox	NADPH oxidase
ONOO-	peroxynitrite
PGE2	prostaglandin E_2
PGG2	prostaglandin G_2
PGH2	prostaglandin H_2
PGI2	prostacyclin
PLA2	phospholipase A_2
PRR	(pro)renin receptor
RAS	renin-angiotensin system
RAAS	renin-angiotensin-aldosterone system
RCT	randomized controlled trials
ROS	reactive oxygen species
SBP	systolic blood pressure
SHR	spontaneously hypertensive rat(s)
TG	triglycerides
TXA2	thromboxane A_2
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelium growth factor
(V)SMC	(vascular) smooth muscle cell
WKY	Wistar Kyoto rat(s)

ABSTRACT

Consumption of polyphenol-rich foods, such as berries, fruits, tea and cocoa has been claimed to exert beneficial effects on cardiovascular health. Wild Nordic berries, e.g. lingonberry (*Vaccinium vitis-idaea*), bilberry (*Vaccinium myrtillus*), cranberry (*Vaccinium oxycoccos, V. microcarpum*) and cultivated blackcurrant (*Ribes nigrum*) are good sources of polyphenols including flavonoids, such as anthocyanidins, proanthocyanidins and flavonols. The aim of this series of studies was to investigate the effects of cranberry, lingonberry and blackcurrant juices on vascular function of genetically hypertensive rats and to clarify how lingonberry affects the blood pressure of spontaneously hypertensive rats and normotensive rats consuming a high-salt diet. We also wanted to determine whether lingonberry juice could exert effects on low-grade inflammation, a phenomenon, which has been related to hypertension and excess salt intake.

The established high blood pressure of spontaneously hypertensive rats became lowered during an eight-week treatment with lingonberry juice. However, more concentrated lingonberry juice was unable to prevent the development of genetic hypertension in young rats. Nonetheless, the endothelium-dependent relaxation of mesenteric arteries was enhanced after eight weeks' treatment with the more concentrated lingonberry juice as was also the endothelium-independent relaxation.

Positive effects of lingonberry juice on inflammatory markers were observed in both rat models. After lingonberry juice treatment, serum levels of both angiotensin II and alkaline phosphatase were lower than in the control groups. Possible anti-inflammatory and anti-thrombotic effects were present due to the reduced gene expression of cyclooxygenase 2 (COX2), monocyte chemoattractant protein 1, p-selectin and vascular cell adhesion molecule 1. These results indicate that inhibition of the renin-angiotensin system together with enhanced nitric oxide production could be possible mechanisms behind the positive effects on blood pressure and vascular function. The lingonberry treatment lowered gene expression of COX2 in the aorta, and increased COX2 protein expression in the kidney cortex *macula densa,* possibly indicating that inducible COX2 had been inhibited whereas the important constitutive COX2 was maintained by lingonberry treatment. Molecular docking studies conducted with flavonoid structures indicated that kaempferol may exert inhibitory effects on COX2.

In summary, in an experimental model of hypertension, long-term treatment with lingonberry juice was able to lower blood pressure and improve vascular function. The effects on RAS and possible ACE1 inhibition together with enhanced nitric oxide bioavailability are potential mechanisms involved in these positive cardiovascular effects. Furthermore, lingonberry possesses anti-inflammatory properties, which may well contribute to its ability to reduce blood pressure and improve vascular function.

1 INTRODUCTION

Cardiovascular diseases are a major cause of death all around the world (Benjamin *et al.* 2018). Almost every second Finnish man and every third woman suffer from increased blood pressure. During recent years, the levels of Finnish adults' diastolic blood pressure (DBP) have started to increase, whereas systolic blood pressure (SBP) has slightly declined. The causes behind the increased DBP values have been suggested to be alcohol, obesity and excess salt intake (Laatikainen *et al.* 2012).

The deterioration of vascular function is one of the first findings in cardiovascular disease and this phenomenon has been linked with hypertension, smoking, hyperlipidemia or diabetes, all of which contribute to premature vascular aging (Guzik and Touyz 2017). Recently, hypertension and vascular dysfunction have been regarded as consequences of inflammatory pathophysiological processes. It has also been postulated that hypertension is a consequence of an imbalance in body homeostasis, a disturbance in important endogenous systems and subsequent physiological errors. The renin-angiotensin system and the kidneys play a major role in the regulation of blood pressure in conjunction with neuronal and hormonal mechanisms.

In addition to medication, lifestyle guidance and consuming a healthy diet are important aspects of both the prevention and the treatment of cardiovascular diseases. A diet containing an abundance of polyphenol-rich berries, fruits and vegetables has been shown to improve cardiovascular health and to decrease the risk of mortality (Rissanen *et al.* 2003; Jennings *et al.* 2012; Kimble *et al.* 2018). Most of the experimental and clinical studies have been conducted with fruits, tea and cocoa. However, wild Nordic berries, such as lingonberry (*Vaccinium vitis-idaea*), bilberry (*Vaccinium myrtillus*), cranberry (*Vaccinium oxycoccos, V. microcarpum*) and cultivated blackcurrant (*Ribes nigrum*) are excellent sources of polyphenols including flavonoids, such as anthocyanidins, proanthocyanidins and flavonols.

The aims of this series of studies were to investigate the effects of cranberry, lingonberry and blackcurrant juices on the vascular function of genetically hypertensive rats. We also wanted to examine the effect of lingonberry juice on blood pressure and inflammatory status as measured with circulating and local vascular markers. We used a salt-loaded rat model to determine if lingonberry could exert effects on kidney function and inflammation. Finally, we examined the actions of the lingonberry juice on an important inflammatory and vasoactive mediator, cyclooxygenase 2 (COX2).

2 REVIEW OF THE LITERATURE

2.1 Regulation of blood pressure

Blood pressure is the hydrostatic pressure in the circulatory system. Two parameters, 1) total peripheral resistance and 2) cardiac output, the latter representing the amount of blood pumped to the aorta in one minute, are responsible for the blood pressure. Blood pressure can be detected as systolic or diastolic blood pressure, diastolic being the period of relaxation and systolic representing the period of contraction. Long-term blood pressure is controlled by several mechanisms involving nerves, kidneys and vasculature.

Vascular tone is controlled by neuronal, hormonal and other circulating agents, as well as by factors released from endothelium acting finally on vascular smooth muscle. Vascular function is a crucial physiological mechanism, where different cell types, receptors, enzymes, hormones, vasodilators and constrictors interact to maintain the balance and homeostasis of the body. Peripheral resistance and blood pressure are mainly determined by the conditions in the small arteries and networks, since flow resistance according to Poiseuille´s law is inversely proportional to the fourth power of blood vessel diameter. Thus, normal functioning of the arteries is essential for maintaining normal blood pressure.

Aging, lifestyle habits and heredity all affect the development of the blood pressure. Excess consumption of alcohol and salt, being overweight and physically inactive as well as stress make an individual susceptible to becoming hypertensive. According to Finnish Current Care Guidelines, blood pressure is categorized as increased if SBP is over 140 mmHg and DBP over 90 mmHg (Hypertension: Current Care Guidelines, 2014). However, in the USA, these categories have been changed in recent years and blood pressure is considered elevated, if SBP exceeds 120 mmHg and DBP 80 mmHg (Whelton *et al.* 2018). Hypertension exposes the individual to serious risks e.g. to coronary artery disease and stroke. The presence of high cholesterol levels, metabolic disorder, insulin resistance and renal dysfunction commonly co-exist with hypertension. Untreated hypertension is a major “silent” risk for death.

Blood pressure is mainly regulated by the sympathetic (adrenergic) nervous system, kidney and body fluid homeostasis. The parasympathetic (cholinergic) nervous system makes a contribution by regulating heart function. Long-term blood pressure is regulated through the kidneys, the Renin-Angiotensin-System (RAS) and the Kallikrein-Kinin-System (KKS).

2.1.1 Sympathetic nervous system

The sympathetic nervous system is responsible for the rapid changes taking place in blood pressure, whereas central nervous system controls the level over longer periods (6-8 h). The vasomotor centre in the brain medulla maintains the appropriate tonus of arteries and regulates the heart rate via parasympathetic signals transmitted through the vagal nerve. The sympathetic nerves innervate organs and blood vessels, except the capillaries. An elevated activity of sympathetic nerves in kidneys increases renal vascular resistance, triggers renin release and promotes tubular sodium reabsorption, all of which contribute to the retention of sodium and water, inducing an increase in arterial pressure. (Hall and Guyton 2011a)

Baroreceptor reflex

The sympathetic barosensitive neurofibrils are regulated by baroreceptors and stimulation of baroreceptors transmits a signal that is received by the central nervous system from where it is then transferred to the autonomic nervous system. This baroreceptor reflex aims to stabilize blood pressure by adjusting the vascular diameter and heart function but persistent hypertension diminishes its sensitivity. Baroreceptors are typically located in the carotid sinus and aortic arch, although a few receptors are also present in other large arteries. Neurons in the *tractus solitarius* in the central nervous system receive the signal transmitted by baroreceptors through glossopharyngeal and vagus nerves and deliver the information to the sympathetic and parasympathetic centers in the brainstem. Signalling in *tractus solitarius* then triggers an inhibition of the vasoconstrictor signals as well as an activation of the parasympathetic center to dilate the arteries, decrease heart rate and to reduce the strength of cardiac contraction. (Guyenet 2006; Lohmeier and Iliescu 2015)

The baroreflex affects the long-term control of blood pressure and it appears to communicate also with renin-angiotensin-aldosterone system (RAAS). The potent vasoconstrictive agent, angiotensin II (Ang II), has detrimental effects on the autonomic nervous system and baroreceptor reflex. For instance, angiotensin II causes a sympathetic neural discharge and increases the density of sympathetic innervation. Furthermore, the activity in the arterial baroreceptor reflex is reduced when baroreceptors in the aortic arch and vagal nerve firing are inhibited by angiotensin II. Vasoconstriction mediated via the α1 adrenergic receptor is increased as the response to noradrenaline is enhanced. Angiotensin 1-7 (Ang 1-7), a newly found component of RAS seems to have opposite effects on the autonomic nervous system, *e.g.* arterial baroreceptor function improves whereas discharges from the sympathetic nerves decrease. Furthermore, Ang1-7 has direct vasodilatory effects via Mas-receptors. (Miller and Arnold 2018)

Alpha- and beta-adrenergic regulation

The catecholamines, adrenaline and noradrenaline, as well as their precursor dopamine are the most important neurotransmitters in the sympathetic nervous system. Adrenaline is secreted from the adrenal gland whereas noradrenaline is mainly released from sympathetic nerve endings. Vasoconstriction occurs when noradrenaline is released from nerve endings into the synaptic cleft. Catecholamine signalling is transduced through G-protein coupled α- and β-adrenergic receptors. Stimulation of α- and β-adrenergic receptors activates inhibitory or excitatory effects, depending on the organs in which they are situated. For example, α-adrenergic receptors induce vasoconstriction in the arteries, but relaxation in the intestine. (Hall and Guyton 2011 b) In the vascular smooth muscle, α-receptor subtypes (α1 and α2) mediate vasoconstriction. The predominant postsynaptic subtype depends on the vascular site, diameter and the individual (Langer and Hicks 1984). Stimulation of β-receptors causes a dilatation of vascular smooth muscle and endothelium-derived vasoactive agents regulate vascular tone locally. It seems that the role of nitric oxide (NO) in β-mediated vasorelaxation is highly dependent on the vascular area and size of the artery (Priest *et al.* 1997). For example, mesenteric resistance arteries are relaxed by NO after stimulation of β_1-adrenoreceptor in rats (Graves and Poston 1993). Beta-adrenergic receptors regulate renin secretion from juxtaglomerular cells in kidneys. The renin-angiotensin system and sympathetic nervous system act in parallel to regulate blood pressure. The sympathetic nervous system also controls to some extent sodium reabsorption in the renal tubules. (Wehrwein and Joyner 2013)

2.1.2 Renin-angiotensin system

Indications of renin-angiotensin system (RAS) were initially discovered by the Finnish physiologist Robert Tigerstedt in collaboration with Per Bergman in 1898 when they detected the presence of renin in kidney lysate (Tigerstedt and Bergman 1898). However, it was almost a century later before the importance of RAS in blood pressure control and fluid homeostasis was understood (Peart 1975). Today, it is known that RAS is an endocrine, paracrine and intracrine system, which is involved in several crucial processes in the body. Traditionally, RAS is known to regulate blood pressure, but it also contributes to inflammatory response, oxidative stress and cognitive function. A local RAS has been found in several organs; heart, arteries, kidneys, adrenal glands, brain, intestine, adipose tissue and eye. The RAS includes several angiotensin peptides and peptidases and at least six receptors. The Angiotensin converting enzyme 1 (ACE1) – Angiotensin II – AT-1R route is involved in vasoconstriction, cell differentiation, regulation of fluid homeostasis and fibrosis. Conversely, Angiotensin converting enzyme 2 (ACE2) – Angiotensin 1-7 – Mas-receptor (MasR) axis acts in an antagonistic fashion to inhibit the effects of angiotensin II. (Fyhrquist and Saijonmaa 2008) Even intraocular pressure is partly regulated by RAS (Holappa *et al.* 2017). Figure 1 illustrates the most important RAS pathways.

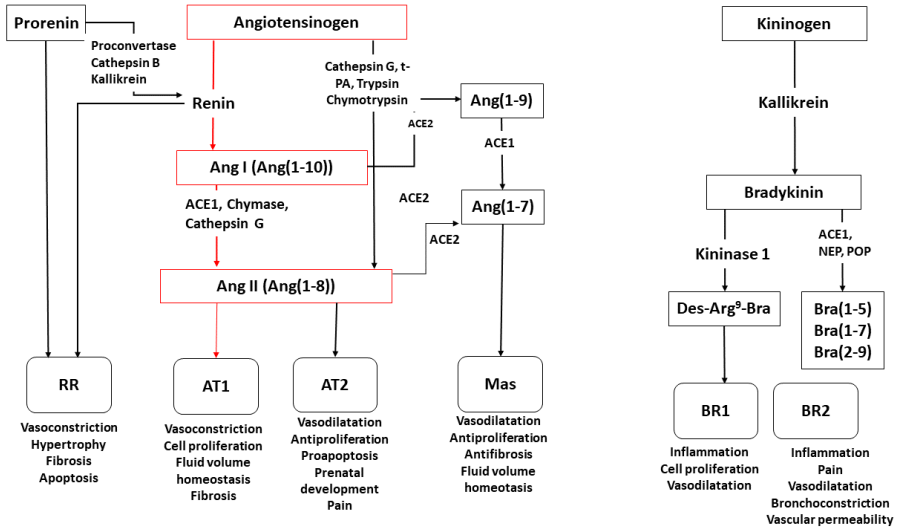


Figure 1. Simplified illustration of renin-angiotensin system and kallikrein-kinin system. RR=(pro)renin receptor, t-PA=tissue-type plasminogen activator, NEP=neutral endopeptidase, POP=prolyl oligopeptidase. Des-Arg-Bra=des-Arg-bradykinin. *Modified from Siltari 2018 with permission.*

Angiotensin I (Ang I), a weak vasoconstrictor itself, is cleaved to angiotensin II (Ang II), one of the most powerful vasoconstrictors in the body, by ACE1. Angiotensinogen is the only precursor for all angiotensin peptides, but its actions are complex. Renin cleaves 10 N-terminal amino acids to produce Ang I from which other angiotensin peptides are formed. This cleavage is the rate-limiting step and the efficacy can be alleviated by domain interactions occurring behind the N-terminal amino acids (Streatfeild-James *et al.* 1998).

Renin and (pro)renin

Renin and its precursor prorenin are produced in the kidneys. Renin is an enzyme which breaks down angiotensinogen (from liver) into Ang I. Prorenin is secreted from the juxtaglomerular cells in the nearby renal glomerulus and is activated by cathepsin β or the proconvertase enzyme in a response to a reduction in blood pressure, lowered sodium balance or activation of central nervous system (Davis and Freeman 1976; Kurtz 2011). The releases of renin and prorenin are mediated by (pro)renin-receptor (PRR) (Nguyen *et al.* 2002). Renin and prorenin can act as vasoconstrictors via PRR. When prorenin binds to PRR, this stimulates intracellular signalling cascades controlling cell proliferation, cycle, survival and angiogenesis. Angiotensinogen cleavage is markedly enhanced when prorenin is bound to PRR expressing membranes; it is a potent form of angiotensin II -independent signalling. (Peters 2017).

The kidney (the juxtaglomerular apparatus) is the main regulator of renin secretion under normal conditions. Renin can be produced also in mast-cells and macrophages. Intracellular calcium and cyclic adenosine monophosphate (cAMP), prostacyclin (PGI2) and prostaglandin E_2 (PGE2) are all able to stimulate renin release. cAMP activity is inhibited by Ang II and calcium-signalling pathway I in renin-producing cells which have been appropriately stimulated (Kurtz and Penner 1989; Edwards and Stack 1993; Kurtz 2011).

ACE1

Carboxypeptidase angiotensin convertase enzyme 1 (ACE1) converts angiotensin I into angiotensin II by cleavage of two amino acids from the peptide's C-terminal end. Various epithelial cells, e.g. proximal tubule epithelium, intestinal epithelium, endothelium and lung capillary cells express ACE1 (Bruneval *et al.* 1986; Culver *et al.* 2017). Somatic ACE1 is a polypeptide with two homologous active sites, N and C (Soubrier *et al.* 1988). ACE1 is not specific for Ang I; it also breaks down other peptides and proteins, such as bradykinin, encephalin, neurotensin and cholecystokinin. The structure of ACE1 is homologous to the bradykinin metabolizing kininase II in the kallikrein-kinin system (Rahimi 2016). Circulating ACE1 is synthetized prominently in lungs, but local production of ACE1 in the kidneys is even five times greater. The levels of kidney ACE1 are significantly increased in several blood pressure and kidney injury models. (Culver *et al.* 2017)

Angiotensin II

Angiotensin II is one of the most powerful physiological vasoconstrictors. It is an octapeptide formed by cleavage of angiotensin I by ACE1 or other enzymes, such as cathepsin G, kallikrein, tonin and chymase. The actions of Ang II are mediated via counteracting AT1- and AT2- receptors (Figure 1). Binding to AT1R stimulates intracellular pathways; calcium channels, NADPH oxidases (NOx) and MAP kinases evoking vasoconstriction, inflammation and fibrosis. The formations of reactive oxygen species and several oxidases i.e. NOx 1, 2, 4 and 5, are stimulated by Ang II. Ang II promotes inflammation by accelerating reactive oxygen species (ROS) production, and increasing the production of cytokines, adhesion molecules and redox-sensitive inflammatory genes. (Montezano *et al.* 2014) Small peptides are cleaved from Ang II, some having vasodilatory and some with vasoconstrictive effects. Ang 1-7 and its close relative alamandine are able to counteract Ang II by acting through the sympathetic nervous system, and inhibiting the baroreflex and inflammation. Ang 1-7 acts through MasR, mediating NO production by inhibition of MAP-kinases, activation of protein tyrosine phosphatase and inactivating the ROS formed by NOx (Montezano *et al.* 2014; Miller and Arnold 2018) .

ACE2

ACE2, an isoenzyme for ACE1, was first described by Donoghue et al. (2000). ACE2 acts as a carboxypeptidase and metalloproteinase (Hooper and Turner 2003). ACE2 has highest affinity for Ang II, but it also hydrolyses other small angiotensin peptides. ACE2 activity is down-regulated in pathological states, such as hypertension and renal disorders (Montezano *et al.* 2014). ACE2 is mainly expressed in the heart, endothelium, testes and kidneys (Donoghue *et al.* 2000), but it has been detected also in other tissues, like intestine, lungs and liver (Tipnis *et al.* 2000; Hamming *et al.* 2004). Interestingly, as well as peptidase activity, ACE2 is a receptor for the SARS coronavirus, but it also protects lungs from lethal lung failure in SARS (Kuba *et al.* 2010).

2.1.3 Kallikrein-kinin system

The kallikrein-kinin system (KKS) participates in blood pressure regulation in addition to RAS. Kinins (bradykinin, kallidin and methionyl-lysyl-bradykinin) are physiologically and pharmacologically active polypeptides, which are released by kallikrein enzymes from body fluids and tissues. Bradykinin was first detected in a snake venom by Rocha and Silva in 1949, when they observed the venom's blood pressure lowering effect. In many respects, KKS functions as a compensatory system to RAS. It causes vasodilatation, regulates sodium excretion and reduces total peripheral resistance. Endothelium-dependent relaxation is enhanced by bradykinin and mediated by NO, endothelium-dependent hyperpolarizing factor (EDHF) and prostacyclin via several mechanisms (Ohlmann *et al.* 1997). Arterial smooth muscle tone is maintained by bradykinin and other kinins. The actions of kinins are mediated by bradykinin-receptors (BR1 and BR2) which are receptors that are produced as needed locally from the endothelial surface (Duka *et al.* 2006). The bradykinin receptors have different roles, with BR1 being more evident in pathophysiological conditions. It has been reported that BR2 expression is decreased in the left ventricles of patients with end state heart failure (Kuoppala *et al.* 2002). In contrast, Liesmaa *et al*. (2005) found that in the endothelium of coronary arteries, BR1 receptors were overexpressed in patients with coronary artery disease and idiopathic dilated cardiomyopathy. ACE inhibitors have shown to potentiate the effects of bradykinin on endothelium-dependent relaxation in bovine and human arteries (Auch-Schwelk *et al.* 1993). Inflammation, chemical and physical stressors activate circulating kallikrein and bradykinin. It has been demonstrated that the actions of bradykinin are dependent on age (Mantelli *et al.* 1995; Siltari *et al.* 2016). For example, Siltari *et al*. (2017) found that in old spontaneously hypertensive rats (SHR), bradykinin is even vasoconstrictive, an effect proposed to be due to vascular inflammation and dysfunction.

2.1.4 Kidneys as a regulator of blood pressure

The kidneys are an important regulator of blood pressure and body fluid and electrolyte homeostasis, as well as excreting metabolites and xenobiotics. Several hormones are secreted and metabolized in the kidneys. The kidneys regulate blood pressure through two different mechanisms: 1) Sodium ion and water excretion (pressure natriuresis) and 2) Renal RAS.

Pressure natriuresis

Extracellular sodium and water concentrations regulate the extracellular fluid volume and urine excretion. Guyton *et al*. (1972) were the first to hypothesize that the kidney controls extracellular fluid volume as a way of regulating the blood pressure. Changes in blood volume and cardiac output affect renal perfusion pressure. In other words, an increase in arterial blood pressure results in increased excretion of sodium and water in urine. In normal situations, the pressure natriuresis feedback system is stable and blood pressure does not change extensively even if short term sodium intake is high, but long-term excessive salt intake leads to kidney stress and an increase in blood pressure. The excretions of water and electrolytes are stringently mediated by glomerular filtration, tubular reabsorption and secretion. There are several active agents capable of changing medullary blood flow and pressure natriuresis e.g. NO, hemoxygenase and the prostaglandins produced by cyclooxygenases. These vasodilators are antagonistic to the vasoconstrictive Ang II and endothelin, as well as to reactive radicals like O_2- and H_2O_2. (Taal *et al.* 2011; Ivy and Bailey 2014)

Kidney RAS and aldosterone

Circulating renin and Ang II enable the blood pressure to remain unchanged independently of sodium intake. Renin release is continuously controlled; the feedback control is maintained by baroreceptors, sympathetic nerves and chloride-sensitive receptors. (Taal *et al.* 2011)

Sodium reabsorption results in increased blood pressure due to increased extracellular volume. Furthermore, there is elevated sensitivity to the actions of Ang II and other vasoconstrictive agents. In addition, Ang II stimulates prostaglandin and cyclic adenosine monophosphate (cAMP) production in order to reduce calcium intake. (Taal *et al.* 2011) Disturbances in intrarenal RAS are important contributors to the pathophysiology of hypertension and renal disease (Kobori *et al.* 2007; Yang and Xu 2017).

The endothelium in the glomerular arterioles, mesangial cells and distal nephrons express ACE1. The enzyme is also expressed in proximal tubules, especially in the brush border of the tubule (Casarini *et al.* 1997). Peripheral vascular resistance is increased due to direct vasoconstrictive effects, sodium reabsorption is accelerated and the secretion of aldosterone is enhanced. (Culver *et al.* 2017)

Aldosterone, a component of renin-angiotensin-aldosterone system (RAAS), is a mineralocorticoid secreted from adrenal cortex. Its main function is to regulate extracellular fluid volume by transporting ions through epithelium. Aldosterone causes a concentration dependent endothelial dysfunction and it has been shown also to be associated with hypertension. Aldosterone has several actions: it accelerates inflammation by promoting the excretion of histamine from macrophages, it induces vascular remodelling (especially with salt) and stimulates ROS. NO availability is lowered due to aldosterone activation of sodium channels. (Briet and Schiffrin 2013; Vanhoutte *et al.* 2017)

Tubuloglomerular feedback and vasoactive agents in glomeruli

Nephron is the functional unit, where glomeruli and tubules act to filter primary urine from blood. In the glomerulus, primary urine, containing water and electrolytes but not proteins, is filtered. The capillary endothelium surrounding glomerulus expresses inducible (iNOS) and endothelial NO synthase (eNOS) (Bachmann *et al.* 1995; Furusu *et al.* 1998) and produces vasoactive agents such as nitric oxide (NO) and endothelin 1 (ET1) (Jourde-Chiche *et al.* 2019). The juxtaglomerular apparatus next to the glomeruli produces renin and Ang II. *Macula densa* (MD) in the juxtaglomerular apparatus is an important site for regulation of renin release e.g. in response to the concentration of chloride in tubular fluid together with a contraction of afferent arterioles. Prostacyclin is released from *macula densa* in states with low tubule fluid chloride concentrations whereas ATP is released when the chloride concentration is high. (Kurtz 2011) Tubuloglomerular feedback regulates the glomerular filtration rate via the glomerular arterioles. Initiation of renin expression starts when sodium- and chloride concentration changes are detected by *macula densa* cells (Taal *et al.* 2011). The level of renin expression parallels the expression levels of nNOS and COX2 in *macula densa* cells as evidence for a connection between renin expression, COX2 and nNOS derived NO (Harris *et al.* 1994; Harris and Breyer 2001; Harris *et al.* 2004).

To summarize, blood pressure is controlled by several mechanisms and systems acting in harmony with each other. The sympathetic nervous system, acting via baroreceptor reflex and α- and β-adrenergic receptors, is generally responsible of rapid blood pressure control. The renin-angiotensin system (RAS), kallikrein-kinin system and kidneys are the main regulators of long-term blood pressure and fluid homeostasis. The components of RAS include vasodilatory and vasoconstrictive agents, such as angiotensin II and angiotensin 1-7 together with their receptors AT1R, AT2R and Mas. Angiotensin II is a powerful vasoconstrictor and it mediates its effects also through inflammatory and oxidative pathways. The kidneys regulate blood pressure mainly by pressure natriuresis and local RAS. Aldosterone secretion from adrenal cortex is associated with RAS and is an important physiological regulator of electrolyte (Na^+, K^+) excretion in the kidneys.

2.2 Vascular function

2.2.1 Arterial structure

Blood vessels consist of three different layers; *tunica intima*, *tunica media* and *tunica adventiti*a (Figure 2). These layers are further divided into different layers. *Tunica adventitia* contains connective tissue, small blood vessels (*vasa vasorum*) and nerves (*nervi vasorum*). Connective tissue is a complex of differently orientated collagen fibres, fibroblasts and elastic fibres. Witter *et al.* (2017) postulated that *tunica adventitia* in fact consists of two parts: *tunica externa*, the compact connective tissue layer and *tunica adventitia*, a loose connective tissue layer which is the outermost part of the vessel. *Tunica media* consists mainly of vascular smooth muscle cells (VSMC), but there are also some collagen fibres, elastic fibres and proteoglycans secreted by VSMC. The innermost part of the vessel, *tunica intima*, is basically formed of endothelial cells and elastic basal lamina, which separates endothelial cells from the vascular smooth muscle cells. According to current knowledge, *tunica adventitia* has an important role in vessel function. Vascular superoxide production, oxidative stress and vascular remodelling in vascular pathophysiology states seem to occur in *adventitia* (Wang *et al.* 1998; An *et al.* 2007). Vessel diameter, or smooth muscle cell function, are modulated by vasoactive agents as well as by the ROS secreted by the endothelial cells (Figure 3).

Arterial smooth muscle

In healthy arteries, smooth muscle cells (SMC) are organized within medial layers in electrically coupled aggregates. The highly plastic and multifunctional nature of SMC enables both vasodilatory and vasoconstrictive actions, which are important in the control of vascular tone and pressure. Dedifferentiation of smooth muscle cells can be triggered by shear stress as well as by the presence of growth factors and cytokines produced by endothelium. In the pathogenesis of vascular disease, dedifferentiation can lead to the smooth muscle cells adopting a migratory and proliferatory phenotype. (Majesky 2016) Phenotype transitions are also possible via Ang II and transcription factors. The consequences of this phenomenon include vascular hypertrophy and the release of vasoconstrictive, inflammatory factors into the vasculature. (Montezano *et al.* 2014)

Vascular diameter is controlled in vascular smooth muscle by phosphorylation and de-phosphorylation of contractile proteins. The Ca^{2+} concentration and calcium signalling are the most important regulators of vascular smooth muscle contraction. Noradrenaline, Ang II, endothelin 1 (ET1), thromboxane A2 (TXA2) together with membrane depolarization or

other chemical or mechanical stimulants can also cause the contraction of VSM. Calcium channels in VSM regulate calcium influx and efflux. (Touyz *et al.* 2018)

The membrane potential of the VSMCs controls the vascular tone via potassium ion (K^+) channel activity and the equilibrium between internal and external K^+ concentrations. The opening of K^+ channels and the efflux of K^+ trigger a membrane hyperpolarization in vascular smooth muscle. Closing of voltage-dependent Ca^{2+} channels and reduced Ca^{2+} entry follow and vasodilatation occurs. There are known to be five different types of K^+ channels in VSMCs: voltage-dependent K^+ channels (KV), Ca^{2+} -activated K^+ channels (BKCa), ATP-sensitive K^+ channels (KATP), tandem two-pore K^+ channels (K2 P) and inward rectifier K^+ channels (K_{ir}) (Dogan *et al.* 2019). The function of the channels and their activation mechanisms are not yet fully understood.

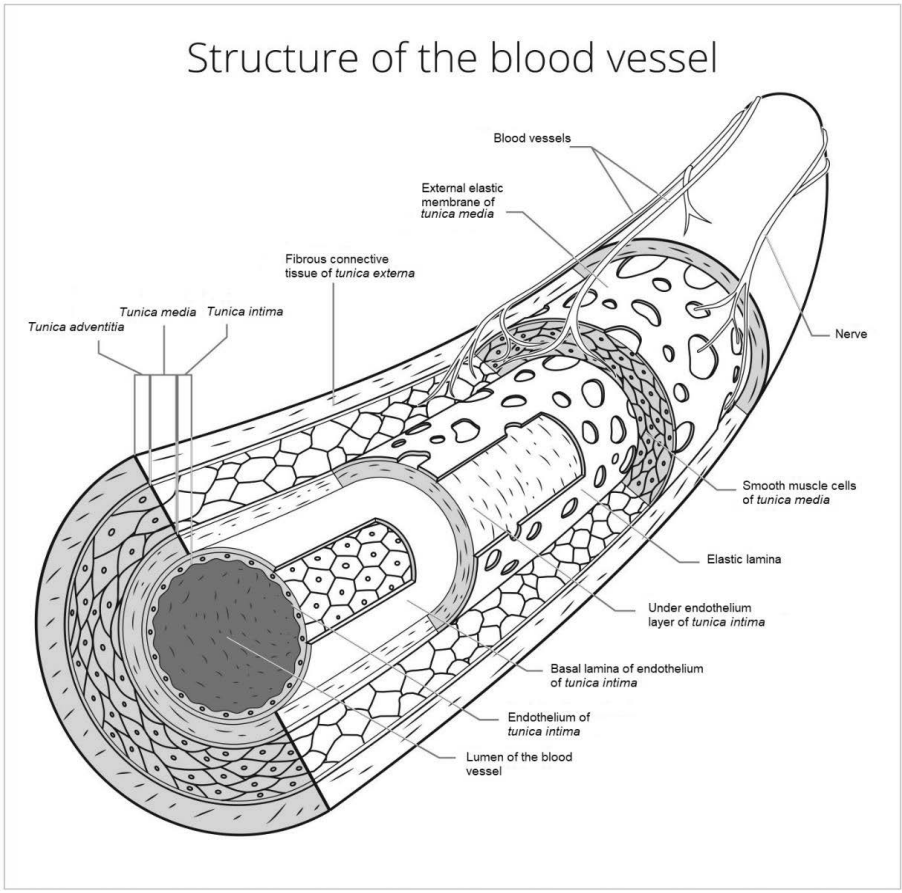


Figure 2. Structure of the blood vessel.

Arterial endothelium

The circulatory system is lined with vascular endothelium (Fishman 1982). In arteries, such as the coronary artery, the innermost layer contains endothelial cells and elastic basal lamina. Endothelium is essential in maintaining circulatory health. Endothelium plays a role as an elastic and permeable barrier between the intravascular and extravascular systems. Macromolecules are transported via vascular smooth muscle and lumen. Endothelial cells form a semipermeable barrier through which macromolecules can cross with different mechanisms, most extensively via vesicular transport or through intracellular junctions between cells or with the help of vesicle fusion. (Ogunrinade *et al.* 2002) Vascular endothelial permeability is mostly regulated by vascular endothelial growth factors (VEGFs) and NOS (Tilton *et al.* 1999). Together with the adrenergic nerves and vasoactive substances, vascular function is locally regulated by endothelial cells secreting vasodilatory and vasoconstrictive substances.

Shear stress caused by circulating blood is an important effector of endothelial permeability (Tarbell 2010). Shear stress affects mainly the endothelial cells, due to its tangential force, whereas cyclic strain modulates the activities also of the other parts of the vascular wall. Several vasoactive substances are released from endothelial cells in response to changes in hemodynamics. Vasodilatation usually is stimulated by increased shear stress and modulated via eNOS activity and NO production, whereas the vasoconstrictor, endothelin-1, tends to be released in states of low shear stress. There is also increased formation by COX1 of the vasodilatory prostanoid, prostacyclin. (Cahill and Redmond 2016)

2.2.2 Vasoactive factors released from endothelium

Several stimuli, like circulating hormones (catecholamines, melanocortin, vasopressin), autacoids (histamine, bradykinin, prostacyclin, prostaglandin E2), cytokines, drugs, chemicals or physical forces stimulate the endothelial cells to produce and release vasoactive agents that modulate vascular relaxation, contraction and permeability (Figure 3). Vascular relaxing factors include NO, prostacyclin, epoxyeicosatrienoic acid, adenosine and C-type natriuretic peptide. Vascular contracting factors are endothelin 1 (ET-1), thromboxane A2, isoprostanes, superoxide anion and Ang II. (Vanhoutte *et al.* 2017)

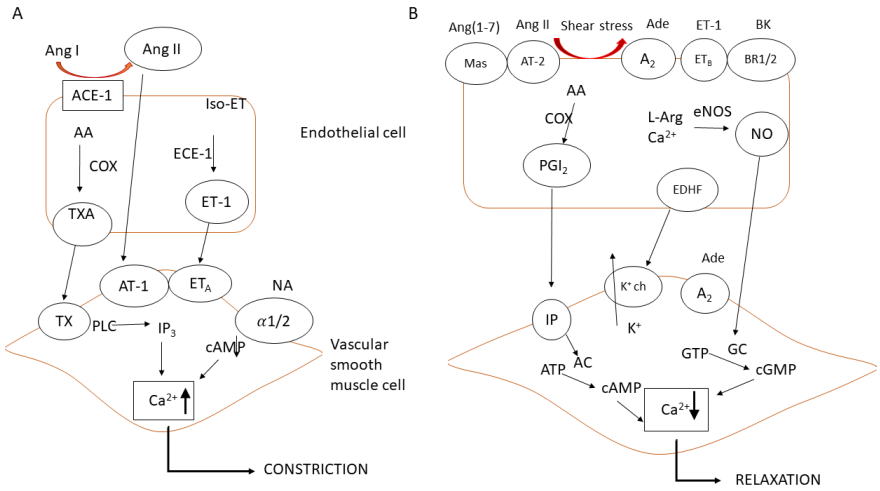


Figure 3. Vasoactive factors released from endothelium and smooth muscle cells during vascular constriction (A) and relaxation (B). Modified from Siltari 2018 with permission.

Nitric oxide

The most important vasodilating substance secreted by endothelial cells is nitric oxide (NO). Furchgott and Zawadzki in 1980 were the first to understand its mechanism. At that time, NO was called endothelium-derived relaxing factor (EDRF). NO/EDRF was identified chemically in 1987 by several researchers (Ignarro *et al.* 1987; Palmer *et al.* 1987). NO is synthetized from L-arginine and oxygen with the help of nitric oxide synthase (NOS) stimulated by chemical and physical stressors. The half-life of gaseous NO is only 6 seconds, so its effects in tissues are strictly local. There are three types of NOS: endothelial (eNOS) and neuronal (nNOS) as well as inducible NOS (iNOS). eNOS is the main regulator of NO in the vasculature and its activation can occur in either a Ca^{2+} -dependent or –independent manner. An imbalance in normal electron flow of eNOS (uncoupling) and a lack of L-arginine or/and (6R-)5,6,7,8-tetrahydrobiopterin (BH4) change the normal NO production into the production of superoxide (O_2-), and also $ONOO^-$, which is formed when NO and O_{2-} react (Moncada and Higgs 2006; Monica *et al.* 2016). Its second messenger, cyclic guanosine monophosphate (cGMP), is formed when intracellular enzyme soluble guanylate cyclase (sGC) converts guanosine triphosphate (GTP) to cGMP under stimulation of NO leading to activation of vasodilating cGMP protein kinase (Ignarro *et al.* 1986). Antithrombotic and anti-atherogenic action in the vasculature can be mediated by NO-sGC signaling but also by COX1 – prostacyclin pathway (Murad 1986; Marcondes *et al.* 2006). A lowered NO level is associated with an increase in platelet aggregation, the contraction and proliferation of smooth muscle, increased expression of adhesion molecules, LDL oxidation and monocyte and platelet adhesion. It is known that cGMP levels are lowered in hypertension and cardiovascular diseases. Diet and physical activity enhance the production of NO, but its level

are reduced by smoking or other oxidative stress states. It has been demonstrated that highly reactive superoxide anions are deleterious to NO. (Monica *et al.* 2016). In contrast, receptor-mediated agonists such as bradykinin stimulate the synthesis of NO via palmitoylation (Robinson *et al.* 1995).

Prostacyclin

In addition to NO, the endothelium can synthetize the vasodilating prostanoid, prostacyclin (PGI2), which acts via cyclic adenosine monophosphate (cAMP) as the second messenger in smooth muscle cells (Moncada and Vane 1978). John Vane received a Nobel Prize in Physiology or Medicine in 1982 for his studies into prostacyclin as well as clarifying the actions of non-steroidal anti-inflammatory drugs (NSAIDs). Prostacyclin is released from blood vessels in both normal and pathological conditions. For example, shear stress *in vivo* and acetylcholine *in vitro* stimulate the formation of prostacyclin. COX synthetizes prostacyclin from arachidonic acid and G-protein-coupled IP-receptors mediate the vasodilatory effects of prostacyclin. Its release from the endothelium of blood vessels is mainly determined by COX1 (Kirkby *et al.* 2013; Mitchell and Kirkby 2019). However, in cases with severe systemic inflammation, COX2 is the main producer of prostacyclin. The actions of prostacyclin are mainly mediated by two receptors, IP receptor and the cytosolic nuclear receptor PPAR β. (Mitchell and Kirkby 2019)

In summary, prostacyclin is a vasodilator, but it also possesses other effects. Prostacyclin inhibits platelet activation and rapidly dilates vessels, and over the long-term, it can affect gene transcription. In the kidneys, renin release from the juxtaglomerular apparatus is partly regulated by prostacyclin (Patrono *et al.* 1982). Prostacyclin possesses also vasoconstrictive effects through the endoperoxides released from endothelium (Vanhoutte *et al.* 2017).

EDHF

Endothelium-derived hyperpolarizing factors (EDHF) are group of vasodilators, which cause vascular relaxation by hyperpolarizing the membrane potential of vascular smooth muscle cells by opening Ca^{2+}-activated potassium channels. Shear stress, agonists (*e.g.* bradykinin and acetylcholine) and intracellular calcium regulate the release of EDHF. Blocking the release NO and/or the prostacyclin formation or their receptors cause compensatory mechanisms, which are thought to be due to EDHF. Endothelium-derived hydrogen peroxide (H_2O_2) has been proposed as one possible candidate of EDHF in humans and animals. However, H_2O_2 possesses a dual role, it is also able to induce COX-dependent thromboxane release and act as an ROS in pathological conditions, although this occurs at higher concentrations than needed for vasodilatation. There are other potential EDHFs e.g. potassium and epoxyeicosatrienoic acid. (Shimokawa 2010; Vanhoutte *et al.* 2017)

Endothelin 1

Endothelin 1 (ET1) is one of the most potent physiological vasoconstrictor substances, initially described by Yanagisawa *et al*. (1988). Endothelial and vascular smooth muscle cells, leukocytes and macrophages synthesize endothelin 1. The vasoconstrictive actions of ET1 are mediated through endothelin receptors. The release of ET1 is potently inhibited by NO. In pathological conditions, ET1 can be also proinflammatory and enhance the proliferation of smooth muscle cells. (Vanhoutte *et al.* 2017)

Endothelium-dependent vascular contraction occurs when vasoconstricting prostanoids are produced in the endothelium by COX. Aging, some diseases (hypertension, diabetes) or obesity all predispose the vascular smooth muscle cells to contract, but this can occur also in healthy and young subjects. (Vanhoutte *et al.* 2017) Cyclooxygenases and thromboxane are discussed in Chapter 2.3.2.

To summarize, the arterial wall consists of three layers, tunica intima, tunica media and tunica adventitia. Tunica adventitia has a major role in controlling vascular function, but the importance of the other layers in controlling vascular homeostasis is becoming more evident. Smooth muscle cells and endothelial cells control vascular viability and function. The vasoactive agents secreted by endothelial and smooth muscle cells can either increase or reduce the vessel diameter. The actions of smooth muscle are contraction and dilation. The most important vasodilating factors are nitric oxide, prostacyclin, adenosine and natriuretic peptide. The most important vasoconstrictive factors include endothelin-1, thromboxane A2, isoprostanes, superoxide anion and angiotensin II. Vascular function is maintained by the balance between these factors. A fluid blood flow and shear stress contribute to vascular function.

2.3 Vascular inflammation and oxidative stress

Vascular inflammation and oxidative stress are key mechanisms underpinning endothelial dysfunction and arterial damage. These states contribute to vascular aging and have been implicated in renal dysfunction, cardiac ischemia, cognitive decline as well macro- and microangiopathy. (Guzik and Touyz 2017)

2.3.1 Inflammatory response and NF-κB

The inflammatory response is mediated by cytokines; of these, nuclear factor kappa B (NF-κB) has a major role. Its activation stimulates the expression of other important cytokines, chemokines, adipokines, cell adhesion molecules and acute phase proteins. Both widely established and unconventional signalling pathways are associated with the activation of NF-κB by different mechanisms. (Liu *et al.* 2017)

Reactive oxygen species (ROS) are highly reactive radicals, which are formed in cellular redox-reactions. Free radicals are very reactive because they have an unpaired electron in their structure. Typically, they are released by inflammatory cells in times of inflammation. An increased amount of ROS attracts more inflammatory cells and this will intensify the effect. ROS act also as signalling molecules regulating vascular stiffness, structure and microcirculation. (Savoia and Schiffrin 2006) In the vasculature, free radicals are often formed via NAD(P)H-oxidases. Ang II can promote the formation of ROS via NOx. The superoxide anion (O_2^-) is a typical ROS and its effect on cardiovascular disease is widely recognized (Nguyen *et al.* 2013). The energy production by mitochondria is impaired by ROS via different mechanisms. This evokes imbalance of calcium homeostasis, ROS formation and modification in redox-signalling. (Sack *et al.* 2017)

Angiotensin II has a major role in vascular inflammation in some tissues and organs, it stimulates the accumulation of monocytes and leucocytes in the subendothelial space of arteries increasing the production of adhesion molecules and chemotactic cytokines as well as inducing smooth muscle cell proliferation and platelet aggregation. In pathological conditions, such as hypertension and diabetes, it is recognized that vascular inflammation promotes atherogenic changes and lipid oxidation. (Montezano *et al.* 2014) Peroxisome proliferator activated receptors (PPAR) act as down-regulators of vascular inflammation, but Ang II inhibits PPARs through NF-kB activation (Tham *et al.* 2002).

2.3.2 Prostanoids and COX enzymes

Lipoxins, leukotrienes, hydroxy-eicosatetraenoic acids, eoxins, isoprostanes, resolvins and prostanoids are included in the eicosanoid family, formed by oxidation of ω3 or ω6 20-carbon fatty acids. Eicosanoids and COX enzymes are widely distributed in the body and are important mediators and regulators of many processes, both for good and bad, including cardiovascular function and inflammation. In the vasculature, prostanoids are the most important eicosanoids. (Mitchell and Kirkby 2019) Cyclooxygenase enzymes catalyze the oxidation of arachidonic acid (AA) released from membrane phospholipids by phospholipase A_2 (PLA2) to prostanoids (Figure 4). Initially, prostaglandin G_2 (PGG2) is formed by the oxidation of AA and further peroxidation yields prostaglandin H_2 (PGH2), both being called also endoperoxides. Prostanoids are formed when specific synthases act on PGH_2. Arachidonic acid release by PLA2 in blood vessels, is the rate limiting step in the prostanoid synthesis (Kirkby *et al.* 2015). Different prostanoids have specific roles in mediating important body responses via different receptors e.g. mediating vasoconstriction, vasodilatation, uterine contraction and participating in allergic reactions. PGE2 acts on EP 1, 2,3 and 4 receptors to trigger vasodilatation, proinflammation, natriuresis and pain. As discussed earlier, prostacyclin acts on IP-receptors regulating vasodilatation, inhibiting platelet aggregation and renin release. Thromboxane A_2 evokes vasoconstriction, bronchoconstriction and platelet aggregation via TPα/β receptors. (Ricciotti and Fitzgerald 2011; Mitchell and Kirkby 2019).

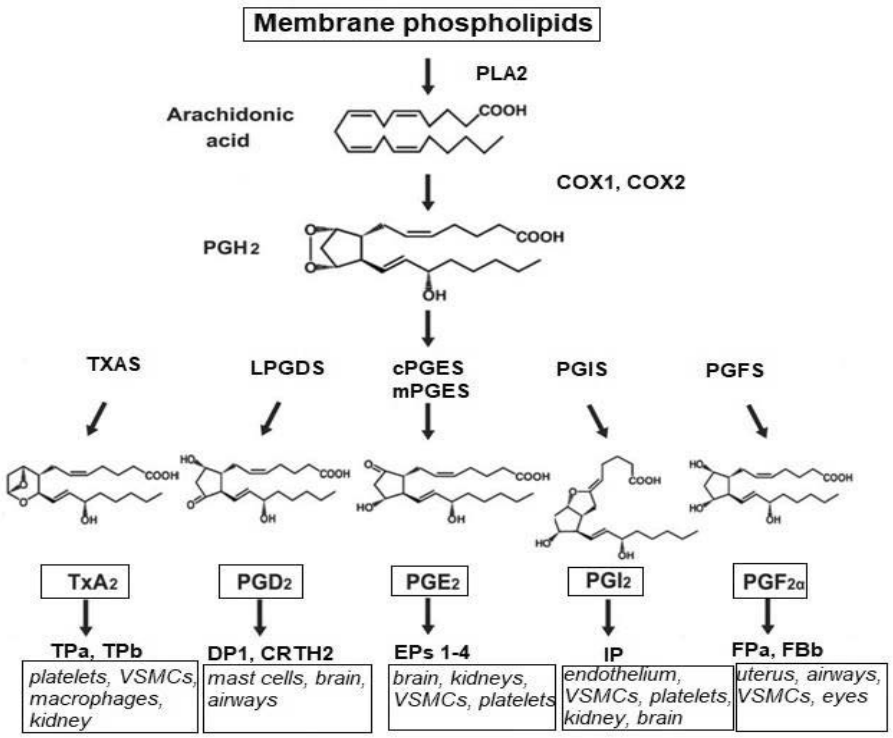


Figure 4. Prostanoid synthesis. Modified from Ricciotti and Fitzgerald 2011.

Two isoforms of cyclooxygenases exist, COX1 and COX2. COX1 was initially identified in 1977 (Hemler and Lands 1977). COX2 was detected by three different groups using different methods in 1989 - 1991 (Rosen *et al.* 1989; Kujubu *et al.* 1991; Xie, W. L. *et al.* 1991). In general, COX1 is constitutively expressed, and COX2 is induced by inflammatory stimulus, like active transcription pathways NF-kβ, nuclear factor of activated T-cells (NFAT) and cAMP (Vane *et al.* 1998). Recently, constitutively expressed COX2 has also been found to be localized in different parts of the body, such as the kidneys, brain, lungs and thymus (Therland *et al.* 2004; Kirkby *et al.* 2013). COX2 has a crucial role in renal function and cardiovascular protection; it takes part in renal function and blood flow and is activated by NFAT in non-inflammatory conditions (Kirkby *et al.* 2018). Renin and sodium release, renal blood flow, blood pressure and renal homeostasis are all controlled by COX2. See (Harris 2006). The importance of COX2 in renal function is evident since deletion of COX2 induces changes in over 1000 genes (Ahmetaj-Shala *et al.* 2015). However, in the endothelium, COX1 seems to be responsible for prostacyclin formation, although in cases with low yields of arachidonic acid, COX2 may be important because it has higher affinity for AA which means it can cause activation when there are lower levels of AA present. (Mitchell and Kirkby 2019) In mouse arteries and in the aorta of SHR and diabetic rats, COX1 is upregulated leading to an enhancement of the vasoconstrictive prostanoid production in the endothelium and

contraction of vascular smooth muscle. COX2 upregulation has been demonstrated in times of oxidative stress as well as in patients with hypertension or diabetes (Garcia-Cohen *et al.* 2000; Shi and Vanhoutte 2008). Up-regulation of COX2 was seen also in coronary arteries of patients with diabetes (Szerafin *et al.* 2006). Inducible COX2 is present during pain, inflammation and fever, and furthermore in cancer, it evokes proliferation, angiogenesis and metastasis of the cancer cells. (Mitchell and Kirkby 2019)

In the kidneys, COX2 regulates renal function and blood flow mediated by NFAT. COX2 is expressed in *macula densa*, cortical thick ascending limb and medullary interstitial cells. Its expression seems to be affected by medullary tonicity, growth factors, water and salt intake, adrenal steroids and cytokines. The collecting ducts seem to be the predominant expression site of COX1. (Breyer and Harris 2001)

Non-steroidal anti-inflammatory drugs (NSAID) are mostly non-selective, inhibiting both COX1 and COX2 although the newest agents are COX2 selective. NSAIDs block prostaglandin synthesis by competing with AA for binding to the catalytic site of cyclooxygenase, thus accounting for their anti-inflammatory actions. At the same time as evoking anti-inflammatory actions, several other processes are also disturbed (Breyer *et al.* 2001). Selective COX2 antagonists have been developed; these drugs are often referred to as COXIBs. At first, the benefits of COXIBs were emphasized, since gastrointestinal ulcers/adverse effects were less common as compared to the old non-selective NSAIDs. Deleterious effects on renal balance caused by COXIBs were subsequently observed, including salt retention, hypertension, hyperkalemia, edema and acute renal failure. Interestingly, Ahmetaj-Shala *et al.* (2015) found that the levels of the physiological endothelial nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA), were significantly increased in mice and humans after administration of a selective COX2 inhibitor (celecoxib) as well as after treatment with a non-selective COX1/COX2 inhibitor (naproxen).

2.3.3 CRP and alkaline phosphatase as diagnostic tools

The levels of C-reactive protein (CRP) as well as those of other acute phase proteins are increased in blood in the long term follow-up in patients with increased blood pressure and blood cholesterol (Ridker *et al.* 2000). CRP (high sensitivity) is a marker for predicting the risk of cardiovascular diseases. The baseline level is around 3 mg/l, when it exceeds that value, the risk for CVD increases (Blake *et al.* 2003). Cortez *et al.* (2016) described that in treatment-resistant hypertensives, if CRP had increased over the median (3.8 mg/l), then this doubled the risk for the patient's condition to deteriorate or for mortality due to CVD.

Tissue nonspecific alkaline phosphatase (ALP) is highly expressed in liver and bone, and it is routinely measured in clinical practise. Interestingly, a linear association has been found between ALP and all-cause and cardiovascular mortality and myocardial infarction as well as

with coronary artery disease in older men (Tonelli *et al.* 2009; Park *et al.* 2013; Wannamethee *et al.* 2013). Furthermore, Perticone et al. (2015) showed that serum ALP values were inversely related to endothelium-dependent vasodilatation in uncomplicated and untreated essential hypertensive subjects. In addition, the negative associations of ALP were attenuated if there was a relatively higher serum phosphorus level, even within the normal range. A positive and independent association between serum ALP level and inflammatory markers, CRP and leukocyte count, was found in adults aged 60 years or older (Seo *et al.* 2019).

To summarize, vascular inflammation like in severe hypertension leads to arterial damage and endothelial dysfunction. Cytokines and reactive oxygen species are present in inflammatory states. Nuclear-factor κB is an important mediator of inflammation and it has a major role also in vascular inflammation acting through different pathways. Angiotensin II stimulates inflammation and the formation of ROS. Prostanoids are members of the eicosanoid-family. Different prostanoids have several physiological effects such as vasoconstriction, vasodilatation, inflammation, natriuresis and pain. Cyclooxygenase-enzymes exist in two isoforms, COX1 and COX2. COX2 is mainly inducible and its levels are increased in inflammatory states. However, also constitutive COX2 exists and it has an important role in the regulation of renal function and blood flow. Non-steroidal anti-inflammatory drugs (NSAIDs) are inhibitors of cyclooxygenases. Serum C-reactive protein (hs-CRP) and alkaline phosphatase (ALP) can be used as diagnostic tools to monitor the extent of vascular inflammation and its associations with the incidence of cardiovascular diseases.

2.4 Hypertension and vascular dysfunction – pharmacological and dietary treatments

Hypertension is a major risk factor for cardiovascular diseases and causes damage to several target organs, like left ventricle hypertrophy (Kumpusalo *et al.* 2001), renal damage (Rodicio *et al.* 1998), retinopathy (Ong *et al.* 2013) and arterial stiffness (O'Rourke 1990). The increased blood pressure stresses the heart and evokes vascular damage, which may contribute to heart attack and stroke (Kumpusalo *et al.* 2001). Target organ damage weakens the prognosis of hypertensive patients (Harbaoui *et al.* 2016). Even small changes in blood pressure matter, e.g. a 10-12 mmHg reduction of SBP and a 5 mmHg reduction of DBP lower the risk of stroke, coronary artery disease and all-cause mortality (Benjamin *et al.* 2018). The classification of systolic and diastolic blood pressure levels and the diagnosis of hypertension as along with the recommendations for treatment according to Finnish Current Care Guidelines are presented in Table 1.

A key feature of hypertension is early vascular aging, which includes remodelling and the premature development of vascular stiffness (Guzik and Touyz 2017). Vascular stiffness and endothelial dysfunction disable normal vascular functioning and is often related to hypertension, arteriosclerosis and metabolic syndrome. Endothelial dysfunction is an important predictor of cardiovascular disease and one of the first steps on the route to atherosclerosis and coronary artery disease (Vanhoutte *et al.* 2017). The protective role of endothelium is lost being replaced by a proatherosclerotic structure (Vanhoutte 1989; 2017). Reactive oxygen species, which are derivatives from oxygen metabolism, play a major role in vascular dysfunction. ROS counteract the effects of NO resulting in reduced NO bioavailability and the increased formation of peroxynitrite (ONOO-) (Incalza *et al.* 2018). The disturbed blood flow increases the reactions between NO and ROS and activates transcription factors (NF-KB), promoting the expression of monocyte chemoattractant protein 1 (MCP-1) and intracellular adhesion molecule 1 (ICAM-1), which are well known as pro-atherogenic agents. (Hsieh *et al.* 2014) The development of lesions in atherosclerosis is affected by shear stress. Good vascular flow with a high shear stress seems to be atheroprotective, whereas low and oscillated flow in vessel segments promotes plaque formation (Berk *et al.* 2002).

Impaired NO bioavailability accelerates compensatory mechanisms and pathways. At first, endothelial-promoted vasodilatation remains almost normal due to the effects of prostacyclin and EDHF. Endothelial dysfunction and further vascular dysfunction are characterized also by the release of ET-1, TXA2, PGH2 and ROS. At some point, NO itself can contribute to vascular dysfunction by activation of soluble guanylate cyclase which promotes the production of cyclic inosine monophosphate instead of cGMP, which then causes a contraction of VSMC instead of their relaxation. (Vanhoutte *et al.* 2017)

Table 1 Classification of blood pressure levels according to Finnish Current Care Guidelines 2014.

Class	SBP (mmHg)	DBP (mmHg)	Recommended actions
Optimal	<120	<80	Control measurement every 5 years
Normal	120-129	80-84	Lifestyle guidance Control measurement every 2 years
High normal	130-139	85-90	Lifestyle guidance BP levels re-evaluation in 4 months Home self-measurement or ambulatory measurement
Mild hypertension	140-159	91-99	Lifestyle guidance BP levels re-evaluation in 2 months Home self-measurement or ambulatory measurement
Moderate hypertension	160-179	100-109	Lifestyle guidance BP levels re-evaluation in 2 months Home self-measurement or ambulatory measurement
Severe hypertension	>180	>110	Lifestyle guidance BP levels re-evaluation in 1-2 weeks Home self-measurement or ambulatory measurement
Hypertensive crisis	>200	>130	Immediate treatment
Isolated systolic hypertension	>140	<90	Lifestyle guidance BP levels re-evaluation in 2 months Home self-measurement or ambulatory measurement

2.4.1 Pharmacological treatment of hypertension

According to Finnish Current Care Guidelines, drug treatment is recommended, if systolic blood pressure is over 140 mmHg and diastolic 90 mmHg despite lifestyle modifications.
The most common drugs used for hypertension are ACE-inhibitors, AT1 receptor antagonists, beta-blockers, thiazide diuretics and calcium antagonists depending on the patient and disease characteristics. Combinations of these drugs can be used, if monotherapy is not effective enough in lowering the patient's blood pressure.

The conversion of angiotensin I to angiotensin II is inhibited by ACE1-inhibitors (*e.g.* captopril, enalapril, ramipril), thus the formation of vasoconstrictive and proinflammatory Ang II is diminished. In addition, bradykinin breakdown is inhibited and serum aldosterone levels decreased. AT1R antagonists (candesartan, losartan, telmisartan) inhibit the actions of Ang II acting directly on the AT1R. Renin inhibitors (aliskiren) are rather new drugs acting at first steps of RAS-activation by inhibiting the formation of angiotensinogen. Calcium antagonists, like dihydropyridines, diltiazem and verapamil block the cellular entry of calcium through voltage-dependent L-type Ca-channels. Thiazide diuretics (*e.g.* hydrochlorothiazide, trichlormethiazide) and the thiazide-like agent (indapamide) inhibit the Na^+/Cl^- cotransporter in the kidneys. Beta-blockers act by blocking the beta1-receptors and are classified in three groups: non-selective beta-blockers e.g. propranolol and timolol (β1+β blocking), selective beta-blockers e.g. atenolol, bisoprolol, metoprolol (β1 blocking) and beta-blockers with supplemental effects e.g. carvedilol, nebivolol (with some peripheral vasodilating effects). (Williams *et al.* 2018)

2.4.2 Non-pharmacological treatment of hypertension

Changes in lifestyle are the first steps in the non-pharmacological treatment of hypertension, and should be adopted by patients with high normal blood pressure. Excess salt (sodium), alcohol, too little physical activity and obesity are risk factors that can be modified. The importance of diet as a risk factor for cardiovascular diseases is emphasized already in early childhood, when the first indications of endothelial dysfunction and cardio-metabolic disturbances in adulthood are evident (Laitinen *et al.* 2017).

Nutrition has an essential role in the prevention and treatment of hypertension and other cardiovascular diseases. An excessive intake of dietary salt (NaCl) is one of the most important blood pressure elevators (Landowne *et al.* 1949; He *et al.* 2013). Although sodium (Na) is essential for human health in maintaining normal physiological conditions, excess salt intake is a risk factor for cardiovascular diseases (Cook *et al.* 2016). For instance, increased

mortality and the risk of coronary artery disease were associated with a high-salt diet, especially in Finnish overweight men (Tuomilehto *et al.* 2001). Blood pressure increases only slightly during aging, if the individual does not adopt a modern lifestyle and his/her salt intake in the diet is minimal. According to the statement of the European Salt Action Network (Cappuccio *et al.* 2018) and Finnish National Guidelines (National Nutrition Council), consumption of dietary sodium should be less than 5 g/day. According to the FINDIET2017 study (Valsta *et al.* 2018), 90 % of adults in Finland consume an excess amount of salt in their diet. Salt affects blood pressure and health through different mechanisms. For instance, excess sodium decreases NO synthesis and increases the production of asymmetric dimethylarginine (ADMA), which inhibits the production of NO (Houston and Harper 2008).

Other electrolytes, potassium (K), calcium (Ca) and magnesium (Mg) lower blood pressure. The intake of these electrolytes is an important part of a healthy diet. In a meta-analysis of 18 randomized controlled trials (RCT), potassium supplementation (≥4 weeks) decreased SBP (4.5 mmHg) and DBP (3 mmHg) (Filippini *et al.* 2017). The effect was seen especially in those subjects with high-sodium consumption, elevated blood pressure with no medication or those with a low intake of potassium from their diet.

The effects of dietary calcium are varied. In the recent meta-analysis conducted by Jayedi *et al*. (2018), the dietary calcium intake was evaluated of participants in eight prospective cohort studies (almost 250 000 individuals). The authors indicated that the risk of the development of hypertension decreased by 11 % in the highest category of calcium intake when compared to the lowest category and 7 % for each 500 mg/d addition to the calcium intake. However, it has been suggested that there could be calcification of the coronary artery due to calcium supplementation and thus the intake of calcium supplements might decrease cardiovascular health (Tankeu *et al.* 2017). The author of the review nevertheless concluded that dietary calcium intake directly from foodstuffs was not a concern. In experimental studies, calcium has decreased blood pressure with the effect possibly being related to smooth muscle function in arteries (Pörsti *et al.* 1992; Mäkynen *et al.* 1994). Magnesium supplementation decreased SBP by 3-4 mmHg and DBP 2-3 mmHg based on the meta-analysis conducted by Kass *et al*. (2012). An interesting review by Borghi and Cicero (2017) searched for evidence-based and clinically relevant blood pressure lowering effects of different nutrients. They observed that there were some nutrients with promising evidence of blood pressure lowering effects e.g. potassium, magnesium, L-arginine, vitamin C, cocoa flavonoids, beetroot juice, coenzyme Q10, controlled-release melatonin and aged garlic extract. A dose-dependence in the anti-hypertensive effect was evident in these studies. Bioactive tripeptides derived from milk have modest blood pressure lowering effects as shown by some meta-analyses with relatively small populations (Xu *et al.* 2008; Cicero *et al.* 2011; Turpeinen *et al.* 2013).

A dietary approach to stop hypertension (DASH) -diet has been developed for the prevention and treatment of hypertension. Appel *et al.* (1997) used the diet for the first time: subjects received the DASH diet rich in fruits and vegetables together with low-fat dairy products with reduced saturated fat or a diet rich in fruits and vegetables with these being compared

against a typical Western diet as the control. Both diets rich in fruits and vegetables decreased blood pressure, but the DASH diet was more effective in reducing SBP by 5.5 mmHg and DBP by 3.0 mmHg. The combination of a low-sodium and DASH diet seems to be effective in the treatment of hypertension (Juraschek *et al.* 2017). The DASH diet is widely used for the treatment of mild hypertension. Basically, diets rich in vegetables, fruits and berries together with good lipid profile, such as the Mediterranean diet and the Nordic diet have beneficial effects on blood pressure and cardiovascular health (Ramezani-Jolfaie *et al.* 2018).

To summarize, hypertension is major risk for cardiovascular diseases, organ damage and arterial stiffness. Even a slight decrease in blood pressure reduces the risk for stroke and other cardiovascular outcomes. Impaired NO bioavailability, vascular inflammation and increased reactive oxygen species lead to endothelial dysfunction and arterial stiffness, triggering compensatory mechanisms to maintain the vascular function. ACE1 inhibitors, AT1 receptor antagonists, thiazide diuretics, calcium-antagonists and beta-blockers are used for the treatment of hypertension. Together with pharmacological treatment, lifestyle habits and diet have a major role in the treatment of hypertension and the restoration of endothelial function. Sodium increases blood pressure and therefore sodium intake (NaCl) should be minimized in order to decrease blood pressure. Other electrolytes, magnesium, calcium and potassium have been shown to possess blood pressure lowering effects.

2.5 Berries in the prevention of hypertension and vascular diseases

Epidemiological and clinical studies have shown that a polyphenol-rich diet is positively associated with cardiovascular health (Mitjavila *et al.* 2013; Tresserra-Rimbau *et al.* 2014; Rienks *et al.* 2017). Polyphenol-rich cocoa has been studied widely and claimed to exert beneficial cardiovascular effects. The European Food Safety Authority (EFSA) has approved a health claim for cocoa flavanols: "Cocoa flavanols help maintain endothelium-dependent vasodilatation, which contributes to normal blood flow" (EFSA Panel on Dietetic Products, Nutrition, and Allergies 2012). Flavonoid rich foods have promising effects on endothelial function, blood pressure, peripheral and cerebral blood flow. (Rees *et al.* 2018) A higher intake of flavonoids from fruits has been associated with a lower risk of myocardial infarction and ischemic stroke in men (Cassidy *et al.* 2016). A diet rich in anthocyanins has been associated also with lowered blood pressure and mean arterial pressure (Jennings *et al.* 2012). In the SUN cohort study with 17000 participants, those subjects with the highest flavonoid-intake had a 47 % lower incidence of cardiovascular events during a ten-year follow-up when compared to those in the lowest intake quartile (Mendonca *et al.* 2019).

The Nordic diet, which is rich in berries, whole grain, rapeseed oil, fish, fruits, vegetables, nuts and low-fat milk-products, has been claimed to be beneficial *e.g.* lowering mean arterial pressure (Brader *et al.* 2014). Berries have not been studied widely and randomized controlled clinical studies are relatively rare. In this literature review, the focus will be on polyphenol-rich berries and their effects on cardiovascular diseases.

2.5.1 Epidemiological studies on berry consumption and health

Epidemiological studies have shown that berry intake exerts positive effects on health. Mursu *et al*. (2014) found that the consumption of berries was associated with a decreased risk of type 2 diabetes in Finnish men. A reduced risk of mortality was associated with a higher intake of berries, vegetables and fruits in Finnish middle-aged men (Rissanen *et al.* 2003). A delayed risk of all-cause mortality was observed to be associated with higher consumption (over 27 times per month) of vegetables, fruits and berries in a Norwegian study, where 10000 men were followed for 40 years and also the mortality due to cancer and stroke was lower in those men with a higher consumption of vegetables, fruits and berries (Hjartåker *et al.* 2015), although no association with berries on their own was seen. Larsson *et al*. (2013) found that berry consumption was inversely associated with the risk of total stroke and cerebral infarction in a ten-year follow-up of 75,000 men and women in Sweden.

2.5.2 Clinical studies

Berry intake has been shown to have positive effects on cardiovascular diseases (Heneghan *et al.* 2018). A Finnish research group found that a 150 g berry mix divided into two portions per day for eight weeks, lowered blood pressure and positively affected the blood lipid profile in middle-aged study subjects (Erlund *et al.* 2008). The berry mixture consisted of bilberry, lingonberry, strawberry, chokeberry and raspberry. A daily polyphenol dose of 795 mg in berry juice (150 g bilberries, 50 g blackcurrants, elderberries 50 g, lingonberries 50, strawberries 100 g and 100 g tomatoes) for five weeks decreased total cholesterol and LDL-cholesterol levels in 50-70 year-old study subjects (Nilsson *et al.* 2017). The authors also found that berry juice enhanced the performance in a memory test when compared to control group.

Tjelle and co-workers (2015) performed a twelve-week randomised double-blinded placebo-controlled study with polyphenol-rich juices in 134 study subjects, aged between 50-70 years. The subjects had either high range but normal blood pressure values or stage 1-2 hypertension. They were treated with 500 ml of 1) commercially available berry juice mixed from red grapes, cherries, chokeberries and bilberries; 2) juice similar to (1) but enriched with polyphenol-rich extracts from blackcurrant press-residue or 3) a placebo juice (polyphenol contents 246, 305 and 76 mg/100 g, respectively). As compared to the placebo group, the SBP was significantly lowered after 6 weeks when groups 1 and 2 were combined (6.9 and 3.4 mmHg, $p<0.01$). In hypertensive subjects, the reductions were greater (7.3 and 6.8 mmHg, $p<0.04$). A small meta-analysis published by Zhu *et al.* (2017) investigated the blood pressure lowering effects of blueberries in clinical trials conducted before 06/2015; overall six randomized controlled trials were included with 204 participants but no significant differences were seen in blood pressures.

Blueberry enhanced endothelial function in subjects with metabolic syndrome in a six-week study but did not affect the 24 h ambulatory measurement of blood pressure (Stull *et al.* 2015). Johnson *et al*. (2015) conducted a study in post-menopausal women with hypertension or pre-hypertension. The subjects consumed blueberry (*Vaccinium virgatum* and *V. corymbosum*) powder for eight weeks. Systolic blood pressure was significantly lowered in the blueberry group at the end of the study (131 ± 17 mmHg) when compared to basal-stage (138 ± 14 mmHg). No significant difference in blood pressure levels occurred in the control group between the beginning and the end of the study. However, pulse wave velocity in the brachial-ankle was lower in the berry group than in the control group, evidence of better elasticity of the arteries. In addition, the level of NO (nitrate/nitrite in plasma) was higher in the blueberry group at the end of the study (15.35±11.16 µmol/L) when compared to the basal level (9.11±7.95 µmol/L). No differences were evident in the control group.

Levels of systolic (-9%) and diastolic (-6 %) blood pressure of overweight subjects were lower (percentages shown in parenthesis) after the eight week consumption of a blueberry drink (corresponding to 350 g of fresh blueberries) than in the control group (-1.5 and -1.2 %, respectively). The blueberry drink also decreased the amount of oxidized LDL and the serum malonaldehyde concentration (Basu *et al.* 2010). A bilberry portion once a day for eight weeks (400 g/d) had a positive effect on several inflammatory factors, i.e. CRP, IL-6, IL-12 and LPS (Kolehmainen *et al.* 2012). Systolic blood pressure or oxidative stress markers of former smokers were not affected by the consumption of chokeberry extract in the study of Xie *et al*. (2017), but the total cholesterol and low-density lipoprotein (LDL) cholesterol levels were lowered (8 % and 11 %, respectively), diastolic blood pressure slightly increased in the chokeberry group when compared to the control group. Earlier, Kardum *et al*. (2015) showed that chokeberry juice lowered blood pressure (24 h ambulatory) in the sympathetically active group (140.8 ± 9.3 mmHg *vs.* 132.3 ± 15.4 mmHg) and lowered the concentration of triglycerides in the blood of sympathetically and parasympathetically active groups after four weeks' consumption, although it is evident that this study was conducted without proper controls. Loo *et al*. (2016) found that consumption of 330 g chokeberry each day lowered daytime diastolic blood pressure slightly (1.7 mmHg) and affected positively on IL-10 and TNF-α, when compared to the placebo group. Recently, the intake of a freeze-dried bilberry supplement (40 g/d) taken with standard medical therapy for eight weeks enhanced the distance that the patients could walk in 6 minutes after an acute myocardial infarction when compared to the patients receiving only medical therapy (Arevström *et al.* 2019). In addition, *ex vivo* oxidized low-density lipoprotein was lowered in the bilberry group when compared to the control group.

RCT trials, investigating blood pressure, vascular function or inflammation with cranberry, blueberry, bilberry, blackcurrant and strawberry are listed in Table 2. No RCTs related to CVD with lingonberry only were found. Sea buckthorn is not listed, since only a few clinical studies examining cardiovascular outcomes have been published in English. However, sea buckthorn has been claimed to have the potential to augment cardiovascular health, as it has been shown to lower blood lipids and platelet aggregation (Yang *et al.* 1999; Johansson *et al.* 2000; Guo *et al.* 2017). Thirty days' intake of sea buckthorn seed oil decreased systolic blood pressure, and also the blood lipid profile was improved (Vashishtha *et al.* 2017).

Table 2. Randomized controlled trials conducted with cranberry, blueberry, bilberry, blackcurrant and aronia.

	Reference	n	Duration	Amount per day	Effects on blood pressure, vascular function or inflammation
Cranberry	(Ruel *et al.* 2009)	30	12 wk	125-500 ml	BP ↔, Plasma MMP9 ↓
	(Dohadwala *et al.* 2011)	44	4 wk	450ml	BP ↔, PWV ↓, FMD ↔
	(Ruel *et al.* 2013)	35	4 wk	500 ml	Alx ↔
	(Novotny *et al.* 2015)	56	8 wk (2x)	240ml	DBP ↓, CRP ↓, TAG ↓
Blueberry	(McAnulty *et al.* 2014)	25	6 wk	250 g *	DBP #↓, ASP ↓, Alx ↑, NK cells ↑
	(Johnson *et al.* 2015)	48	8 wk	22 g	SBP ↓, DBP ↓, PWV ↓, NO ↑
	(Stull *et al.* 2015)	44	6 wk (2x)	45 g	BB ↔, RHI ↑
	(Curtis, P. J. *et al.* 2019)	115	6 m	150g	FMD ↑, Alx ↑,cGMP↓, PWV↔, BB ↔, NO ↔
Bilberry	(Karlsen *et al.* 2010)	31	4 wk		hsCRP ↓, IL-6↓, IL-15 ↓, INF-δ ↓ TNFα ↑
	(Kolehmainen *et al.* 2012)	27	8 wk	400 g	Inflammation score ↓
	(Habanova *et al.* 2016)	36	6 wk (3x)	150 g	BP ↔, (Lipid profile enhancements)
Blackcurr	(Khan *et al.* 2014)	66	6 wk	250 ml	BP ↔, FMD ↑,F2 isoprostane ↓
	(Dalgård *et al.* 2009)	48	28 d	500 ml	CRP ↓, IL-6 ↔
Strawberry	(Basu *et al.* 2010)	27	8 wk	500 g*	BP ↔, tCHOL ↓, LDL ↓, VCAM ↓
	(Basu *et al.* 2014)	60	12 wk	250 g* 500 g*	BP ↔, LDL ↓, MLD ↓
	(Amani *et al.* 2014)	36	6 wk		DBP ↓
	(Feresin *et al.* 2017)	20	8 wk	25 g# 50 g#	BP ↔, PWV ↔
Choke.	(Loo *et al.* 2016)	37	8 wk	300 ml	DBP ↓

MMP9=matrix metalloproteinase, PWV=pulse wave velocity, FMD=flow-mediated dilatation, Aix=augmentation index, TAG=triacylglycerol, ASP=aortic systolic pressure, RHI=reactive hyperemia index, IL=interleukin, INF=interferon, TNFα=tumor necrosis factor α. *powder, corresponding amount of berries, #amount of powder. 2x/3x = daily portion. ↓/↑ =p<0.05, ↔ = p=ns.

2.5.3 Effects of lingonberry on cardiovascular health

There are rather few clinical studies conducted with lingonberries and none of them have focused on vascular diseases. Instead, lingonberries have been evaluated in association with blood glucose and glycemia. A strong hyperglycemic profile in response to sucrose was significantly improved at two hours after ingestion of lingonberry puree (150 g) with 35 g added sugar when compared to sucrose diluted in 300 ml of water in healthy women in a randomized controlled meal study conducted by Törrönen *et al*. (2012). The effect of lingonberry nectar (300 ml, 50 % of berry) was slightly milder. Similar effects were seen in another study, where glucose and fructose were used instead of sucrose, to mimic the conversion of sucrose into glucose and fructose in solutions with acidic pH values (Törrönen *et al.* 2017). In both studies, lingonberry affected the insulin response, but it was not able to clearly lower the glucose level in blood. Furthermore, when lingonberry (150 g) was served with wheat bread (50 g of starch), the insulin response was lowered when compared to control meal (the same bread and 50 g cucumber with water) (Törrönen *et al.* 2013). The sugars present in the lingonberry itself did not seem to increase blood glucose, probably due to compensatory effects of polyphenols and/or fibre (Linderborg *et al.* 2012).

Most studies on health effects of lingonberries have been conducted in animals. In mice, lingonberry prevented weight gain and the development of fatty liver due to feeding a high-fat diet (Heyman *et al.* 2014). Matziouridou *et al.* (2016) found that the vascular plaque size in APO $E^{-/-}$-mice was reduced and the amount of triglycerides in blood was decreased, when food was fortified with 44 % (weight) lingonberry for eight weeks. In the same study, lingonberry also altered the composition of the gut microbiota. Fatty liver formation and diabetes were studied in C57BL/6-mice, which were fed a high-fat diet and dried lingonberry, bilberry, blackcurrant or acai berries for 13 weeks (Heyman-Linden *et al.* 2016). Lingonberry, bilberry and blackcurrant diminished the effects of the high-fat diet, such as fatty liver formation and disturbances in fat metabolism by reducing low-grade inflammation and acute phase reactions. Interestingly, acai-berries increased the formation of fatty-liver. Beneficial effects on lipids were seen also with lingonberry anthocyanin extract, when mice with induced hypercholesterolemia were given the extract for 10 weeks (Zhang *et al.* 2019). The lingonberry anthocyanin extract reduced the visceral adipose content, total cholesterol and LDL-cholesterol levels. Body mass and daily food intake were also lower and HDL-cholesterol level higher when compared to the control group. In streptozotocin-induced diabetic mice, lingonberry extract was able to restore the density of purinergic receptors and decrease the oxidative stress related to diabetes (Reichert *et al.* 2018). The extent of a renal injury was diminished in ischemia-perfused Sprague-Dawley rats after three weeks' treatment with lingonberry juice and also the level of injury-induced inflammation was decreased (Isaak *et al.* 2017). Interestingly, when lingonberries from two different origins were tested in mice consuming a high-fat diet, only lingonberries from one location were able to improve glycemia in the mice, but berries from both sites were able to improve liver function and inflammation when compared to the control group (Al Hamimi *et al.* 2017). The anti-

oxidative actions of lingonberry have been investigated mostly in *in vitro* studies. Lingonberry polyphenols are able to donate electrons and thus exert antioxidant activities by chelating and quenching free radicals (Zheng and Wang 2003; Määttä-Riihinen *et al.* 2005; Drozdz *et al.* 2017).

To summarize, berries and other polyphenol-rich diets can enhance cardiovascular health. Berry intake has been associated with lowered blood pressure, improved vascular function and has exerted a beneficial effect on the blood lipid profile. Bilberry, blueberry and strawberry have been the most extensively studied berries in this respect and their positive effects on blood pressure, low-grade inflammation and vascular function have been reported in randomized controlled clinical trials. However, more evidence should be gained with controlled amounts of polyphenols. Only a few clinical studies have been conducted with lingonberries. In those studies, lingonberry improved the glycemic profile after consumption of sugars or starch. In preclinical studies conducted in experimental animals, lingonberry treatment has been associated with positive effects on the blood lipid profile, fat metabolism, inflammation and oxidative stress.

2.6 Polyphenols in berries

Polyphenols are aromatic chemicals and secondary metabolites synthetized by plants to combat different threats, like UV-radiation and pathogens. Flavonoids and different phenolic acids are some of the most common groups of polyphenols. About 8000 polyphenols have been identified and 4000 of them are classified as flavonoids. In plants, polyphenols are usually present as glycosides or acylated in sugars. The classification of polyphenols can be made by their chemical- or bioactive structures or origin. See (Tsao 2010). Polyphenols can be roughly divided into flavonoids, and non-flavonoids. Examples of flavonoid classes and subclass are listed in Table 3 and non-flavonoid polyphenols in Table 4 (Manach *et al.* 2004; Tsao 2010; Goszcz *et al.* 2017). There are some seasonal and growth place variability in the polyphenolic contents of berries, for example, the anthocyanin amount seems to be dependent on growth conditions, with the amount being highest at low temperatures (Hykkerud 2018).

2.6.1 Flavonoids

The basic structure of all flavonoids is a three-phenol ring C6-C3-C6, diphenylpropane. Flavonoids are classified in subgroups according to their heterocyclic C-ring. Usually, flavonoids are present in plants as glycosides. Bioactivity is affected by the structure and glycosylation state. The most common flavonoids (Table 3) are flavones, flavonols, flavanones and flavanoles (3-hydroxy derivate of flavanones), which are found in most of the plants. The largest subgroup of polyphenols consists of flavones and their 3-hydroxy derivate flavonols. For instance, two flavonols, quercetin and kaempferol, have about 300 different glycoside structures. Flavanols (flavan-3-ols), so-called catechins have two chiral centers and thus four different stereoisomers i.e. epicatechin is a *cis*- and catechin is a *trans*- isomer. Both forms have also stereoisomers, (+)-epicatechin, (-)-epicatechin, (+)-catechin ja (-)-catechin. Polymers consisting of epicatechins and catechins are called proanthocyanidins. Further anthocyanins are formed when proanthocyanidins are cleaved under acidic conditions. Proanthocyanidins, so-called condensed tannins, are oligomers consisting of 2-11 flavanols. A- and B-type proanthocyanidins differ by the bonds linking the flavanol units. Flavanols and proanthocyanidins are able to act as strong antioxidants and the ability seems to correlate with the polymerization rate. Anthocyanidins occur most often as glycosides, i.e. anthocyanins. The red, blue and purple colours in plants are based on anthocyanins, with most common being cyanidin, delphinidin and pelargonidin. In acidic conditions, anthocyanins are bright red in colour, whereas in more neutral conditions they are blue. In addition, hydroxylation, aromatic rings and glycosylation affect colour formation. (Manach *et al.* 2004; Tsao 2010)

Table 3. Flavonoid classes and example of compounds and common dietary source.

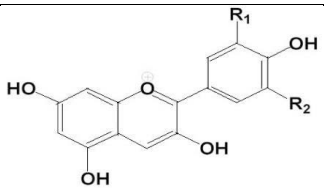
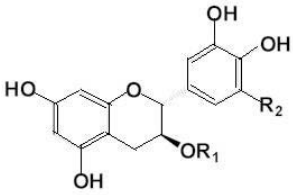
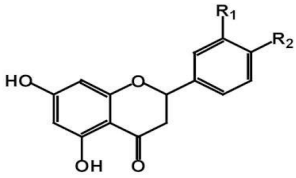
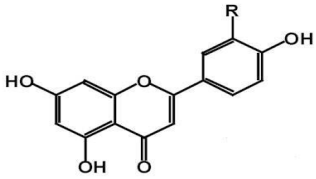
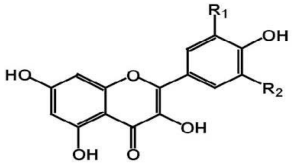
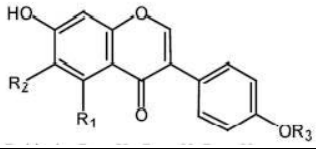
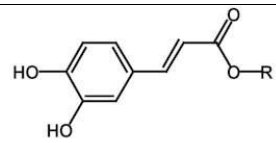
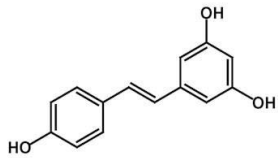
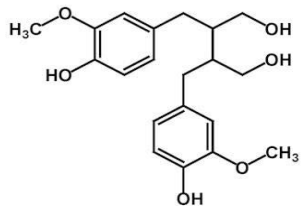
Flavonoid class	Subclass	Example of these compounds	Dietary Source
Anthocyanins	Cyanidin (R_1=OH, R_2=H) Delphinidin ($R_{1,2}$=OH) Pelargonidin ($R_{1,2}$=H)		berries, black beans, eggplant, red wine
Flavan-3-ols, Flavanols	(+)-Epicatechin ($R_{1,2}$=H) Gallocatechin (R_1=H, R_2=OH) Procyanidins (condensed tannins)		tea, hazelnuts, berries
Flavanones	Naringenin (R_1=H, R_2=OH) Hesperidin (R_1=OH, R_2=OCH_3)		citrus fruits, vegetables
Flavones	Apigenin (R=H) Luteolin (R=OH) Baicalein		fruit, vegetables
Flavonols	Quercetin (R_1=H, R_2=OH) Kaempferol (R_1=H, R_2=H) Myricetin ($R_{1,2}$=OH)		onions, berries, chili peppers, broccoli
Isoflavonoids	Genistein (R_1=OH, $R_{2,3}$=H) Daidzein ($R_{1,2,3}$=H)		soybeans

Table 4. Non-flavonoid polyphenol classes with example compounds and common dietary source.

Class	Subclass	Example of compounds	Dietary Source
Phenolic acids	Chlorogenic acid (R=5-quinoyl) Caffeic acid (R=H) Hydrolysable tannins		Berries, grape, coffee, tea, eggplant
Stilbenes	Resveratrol		Red wine, berries, grapes, cacao, peanuts
Lignans	Secolariciresinol		Flax, sesame, grains, berries

2.6.2 Lingonberry and its polyphenols

Lingonberry, *Vaccinium vitis-idaea,* is the most high-yielding forest berry in Finland. According to the National Institute of Health and Welfare database, lingonberry contains energy amounting to approximately 56 kcal/100 g. It is rich in vitamin E (α-tocopherol) and there are also A-, B- and K-group vitamins (National Institute for Health and Welfare, Nutrition Unit. Fineli.). Minerals potassium, calcium, magnesium, phosphorus are present and also iron and zinc can be detected in trace amounts. Linoleic, α-linolenic fatty acids and sterols are also present in lingonberries. The total fibre content is 2.6 g/100 g and the sugar content is 8.2 g/100 g, most of which are fructose and glucose. Based on food components and conditions assessed by Regulation (EC) No 1924/2006 of the European Parliament and of the Council on Nutrition and Health about claims made on foods, the following nutrition claims can be made about lingonberry: high fibre, low fat, saturated fat free, low sodium and natural source of vitamin E.

Lingonberries contain several complex phenolic compounds. Catechins, epicatechins and their polymers, A- and B-type proanthocyanidins have been identified (Ek *et al.* 2006, Hellström *et al.* 2009). A-type proanthocyanidins are more abundant in Finnish lingonberries; their amounts (298-548 mg/100 g) seem to be highly dependent on growing conditions, seasonal differences, state of maturity and harvesting conditions (Hellström *et al.* 2009).
The anthocyanins found in lingonberries were cyanidins, 77 and peonidins 0.6 mg/100g of fresh weight (Koponen *et al.* 2007). Häkkinen *et al*. (1999) found only quercetin, 74 and 146 mg/kg, whereas quercetin, kaempferol and myricetin were detected in Finnish lingonberries grown in different places.

In lingonberries from western Finland, 1-O-benzoyl-b-glucose was the most common phenolic (41 %) analysed (Tian *et al.* 2017). One quarter of the phenolics consisted of flavan-3-ols including (+)-catechin (22%) and (-)-epicatechin (3%). Cyanidin-glycosides were the most common anthocyanins (11 %) but also A-type procyanidin trimers and B-type dimers were found (9 %). In addition, some flavonol glycosides and derivatives from hydroxycinnamic acid were detected.

In another rather recent study with Romanian lingonberries, anthocyanin compounds bonded with an ethyl-bridge were found; cyanidin-pentoside and cyanidin-acetylhexose (Bujor *et al.* 2018). A total of 120 different phenolic compounds was identified in lingonberries.

Stem and leaves were richer in phenolics than the berries (Bujor *et al*. 2018). Most of the phenolic compounds in stems and leaves were flavanols (72-71 % and 27-42 %, respectively) whereas in the berries, the amount of flavanols was about 30-36 %. Some oligomeric flavanols were found e.g. eight B-type dimers, nine B-type trimers, five A-type dimers and four A-type trimers. Monomeric flavanols (+)-catechin and (-)-epicatechin were found in all

parts of the plants. Gallocatechin and -(-)-epigallocatechin were found from all parts of lingonberry. In addition, four different cinchonines were found. In berries, flavanol glycosides accounted for 7-9 % of total weight, e.g. 18 different quercetin-glycosides were found, as well several kaempferol glycosides. Acids, like hydroxycinnamic acid, caffeolytic acid and p-coumarinic acid represented 2-3 % of the lingonberry polyphenols.

In addition to flavonoids and phenolic acids, lingonberries contain also lignans and stilbenes. The lignans that have been detected in Finnish lingonberries include lariciresinol, pinoresinol (586 µg/100g) and secoisolariciresinol (19 µg, 586 µg and 140 µg/100g of fresh weight, respectively) (Nurmi *et al.* 2010). Resveratrol was the only stilbene found in lingonberries, when resveratrol, pterostilbene and piceatannol were found in *Vaccinium*-berries (Rimando *et al.* 2004). The resveratrol concentration was highest in lingonberries (588 µg/100g), when compared to the corresponding concentrations in other berries.

To summarize, dietary polyphenols are phenolic compounds categorized roughly into flavonoids and non-flavonoids. Flavonoids, about 4000 different compounds are further subdivided to anthocyanins, flavanols, flavanones, flavones, flavonols and isoflavonoids. Non-flavonoid compounds are phenolic acids, stilbenes and lignans. Lingonberry grows widely in Nordic forests, it is rich in polyphenols and it contains also vitamins, minerals and other nutrients and fibre. Lingonberry contains complex polyphenols e.g., anthocyanins, catechins, epicatechins, procyanidins and flavonols. In addition, lignans and stilbenes are found. The polyphenol content differs depending on growth conditions, with some types of polyphenols being more sensitive to changes.

2.7 Bioavailability and safety aspects of polyphenols

The bioavailability of polyphenols is the key question when the health effects of polyphenols and flavonoids are examined. The metabolism of these bioactive compounds are not yet fully understood. The most abundant polyphenols in food might not be the one that are dominant in tissues and circulation. Usually, the bioavailability of polyphenols is rather low and their metabolism is extensive. The active compound behind the actions of the flavonoids is usually the conjugated metabolite digested from intestine or some other chemical form produced by the gut microbiota (Williamson et al. 2018).

2.7.1 Bioavailability and food matrix interactions

The first steps of the absorption of polyphenols are the cleavage of the sugar molecule and the diffusion of the phenolic aglycone to intestinal absorptive cells, enterocytes in the duodenum and the proximal half of jejunum. Glucuronidation, methylation or sulfonation of the aglycons occurs in phase II metabolism before the compounds gain access to the circulation as sulphate, glucuronide and/or methylated metabolites. From the circulation, these metabolites can return to small intestine lumen or be distributed to liver for further metabolism. The return to small intestine is possible because of enterohepatic transport into the bile (enterohepatic circulation similarly as occurs for some drugs). The microbiota in the large intestine and colon can cleave polyphenols and metabolites not absorbed in small intestine to produce phenolic acids and hydroxycinnamates for further degradation. (Del Rio *et al.* 2010). Different colonic microbiota species, such as *Lactobacillus, Eubacterium, Bifidobacterium* and *Clostridium* are now appreciated as having an impact on polyphenol metabolism. For instance, kaempferol is transformed to 2-(4-hydroxyphenyl)propionic acid by *Clostridium orbiscidens* and catechin to 3-(3-hydroxyphenyl)propionic acid or 5-(3',4'-dihydroxyphenyl)-*γ*-valerolactone by *Clostridium coccoides* or *Bifidobacterium spp.* (Marin *et al.* 2015) Correspondingly, the presence of polyphenols can alter the gut microbiota. For instance, bifidobacteria and lactobacilli become enriched in the gut after polyphenol consumption (Sheflin *et al.* 2017). There seems to be heterogeneity in the urinary excretion of polyphenol metabolites, e.g. the excretion of γ-valerolactones was higher in young (26 ± 6 years) than in older people (70 ± 4 years) when differences in ADME of cocoa flavanols were studied (Rodriguez-Mateos *et al.* 2015). In the same study, slight differences were seen in plasma levels of epicatechin metabolites, and the authors suggested that the normal age-related decline in renal function might affect plasma levels of glucuronidated and sulfated metabolites.

Manach *et al*. (2005) reviewed the bioavailability 19 major polyphenols from 97 different studies. They noted that gallic acid and isoflavones were the best absorbed polyphenols, followed by catechins, flavanones and quercetin glucosides. Proanthocyanidins, galloylated

tea catechins and anthocyanins were more poorly absorbed. There were also major differences in the kinetics of the different polyphenol classes. Anthocyanins might have better bioavailability than thought previously. A new technique with isotopic labeling has detected more metabolites of cyanidin-3-glucoside than when the blood was assayed with older techniques (Czank *et al.* 2013). Cyanidin-3-glucoside (C3G) pharmacokinetics was studied (in 8 healthy males) by Ferrars *et al*. (2014). C3G and its degradation products, phloroglucinaldehyde (PGA) and protocatechuic acid (PCA), were found in the serum together with 13 different metabolites of PCA and one PGA metabolite, with hippuric acid and ferulic acid being the major metabolites. Maximal concentrations were attained between 2 to 30 h after consumption, and elimination half-lives ranged between 0.5-96 h. Lehtonen *et al*. (2009) investigated cyanidin 3-O-galactoside anthocyanin and its metabolites obtained from lingonberries. Healthy subjects received 300 g of lingonberries as a part of their breakfast, with the metabolites being detected from urine samples by uHPLC-MS/MS. Maximal excretion occurred at 4 to 8 h after meal. Methylated (peonidin galactoside) and glucuronidated (cyanidin glucuronide) metabolites as well intact cyanidin 3-O-galactoside were found.

The food matrix affects the bioavailability of the berry polyphenols. Proteins, lipids and carbohydrates and polyphenols have different interactions either enhancing or decreasing each other's bioavailability. Interactions between proteins and polyphenols are often reversible, noncovalent and hydrophobic. Proteins might prolong the polyphenol stability whereas the dietary fibre and carbohydrate digestion can be slowed down. (Kardum and Glibetic 2018).

2.7.2 Safety issues

Safety issues surrounding berries have been discussed based on safety data related to certain polyphenols, like anthocyanins. The safety of lingonberry alone has not been evaluated in the literature. In 2013, the European Food Safety Authority (EFSA) has made a safety re-evaluation of anthocyanins used as a food additive (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) 2013). EFSA stated that there was not enough available toxicological data for setting a numerical acceptable daily intake (ADI) for anthocyanins. According to toxicological studies conducted on animals administered anthocyanins from currants, blueberries or elderberries, no acute toxic effects occurred when the intake was 20 mg/kg/d in rats and 25 mg/kg/d in mice (Wallace and Giusti 2015). After exposure for either 15 or 90 days, there were no toxic effects with doses >3 g/kg/d in guinea pigs and rats and in generation studies, 9 g/kg/d in rats, mice and rabbits did not evoke any toxic effects over 3 generations. In mice, rats and rabbits, acute oral toxicity has been assessed by EFSA. An extract from currants, blueberries and elderberries which is rich

in cyanidin, petunidin and delphinidin was studied. The values of the LD50 (lethal dose 50 %) for mice and rats were assessed as 25 000 and 20 000 mg/kg of body weight. Anthocyanins are used as a food additive (food colour) with an e-code E 163. Maximum permitted levels (MLPs) according to the Commission Regulation (EU) No 1129/2011 are listed in EFSAs Scientific Opinion. In most foods, anthocyanins can be added as a *quantum satis* principle (the amount enough), with few restriction/exceptions.

Several manufacturers want to produce synthetic polyphenols and commercialize those as food supplements. The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) has made an evaluation of synthetic trans-resveratrol as a novel food. The conclusion of the panel was that a daily dose up to 150 mg/day as a supplement for adults would be safe under the few conditions assessed. Diarrhea and other gastrointestinal symptoms might occur when the daily dose of resveratrol exceeded 1000 mg. The panel noted that the sulphated metabolite of trans-resveratrol might inhibit cytochrome P450 enzymes (CYP) in humans and thus medicines metabolized through CYP2C9 could undergo interactions with trans-resveratrol. It is becoming more evident, that P450 enzymes (the family including CYP2C9) interact with polyphenols. Interactions between polyphenols and CYP3A4, an enzyme regulating gut and liver metabolism of xenobiotics and drugs, have been investigated recently. According to the review conducted by Basheer and Kerem (2015), flavonols such as kaempferol and quercetin as well as many other flavonoids and the non-flavonoid resveratrol can inhibit CYP3A4. In contrast, tangeretin (a citrus flavone) was found to activate the enzyme. Inhibition of CYP enzymes may elevate the plasma concentration of drugs metabolized by those CYPs and thus accentuate the effects of the drug and increase the possibility of adverse effects (Pelkonen *et al.* 1998). For example, resveratrol has been shown to alter the pharmacokinetic properties of diclofenac (an NSAID) by elevating its maximum plasma concentration, possibly due to CYP-inhibition (Bedada *et al.* 2015).

To summarize, polyphenols are commonly metabolized by glucuronidation, sulphonation or methylation. The colonic microbiota has a major effect on bioavailability of the polyphenols and conversely, polyphenols can alter the gut microbiome. This can lead to differences in the bioavailability of the polyphenol between individuals. It can be concluded that the health-promoting effects of polyphenol-rich foods are the sum of different types of polyphenols and their interactions with gut microbiota and individual characteristics. Polyphenols are metabolized rather rapidly, within a few hours, but also after 36 h of digestion, some metabolites have been detected in plasma and urine. The consumption of polyphenol-rich food safety has not been studied. Dietary polyphenols might affect drug metabolism through the CYP-family.

3 AIMS OF THE STUDY

Nutrition plays a major role in the prevention of hypertension and endothelial dysfunction starting from early childhood and lasting throughout the individual's lifetime. Berries and fruits are rich in polyphenols. Polyphenols are naturally occurring phytochemicals present in plants; some of these compounds have been shown to be associated with improved cardiovascular health. The molecular structure of aromatic polyphenols enables these compounds to act as radical scavengers and enzyme inhibitors, which might be the mechanisms behind their cardiovascular effects.

In this project, the effects of polyphenol-rich berries on vascular function and hypertension were explored. The first part of this study was to evaluate the effects of cranberries, lingonberries and blackcurrants on endothelial function of mesenteric arteries. The second part of this project investigated the effects of lingonberries on blood pressure and on low-grade inflammation. Finally, the mechanisms behind the vascular effects of lingonberries were investigated.

The specific aims were:

1) to study whether cranberry, blackcurrant and lingonberry juices could inhibit the development of vascular dysfunction.

2) to investigate if lingonberry juice affects the development of hypertension and whether elevated high blood pressure could be lowered by long-term treatment with lingonberry juice.

3) to explore the mechanisms contributing to the prevention of vascular dysfunction and hypertension by lingonberry juice and how the inflammatory status could be affected by berry juice.

4) to determine the effects of lingonberry juice on blood pressure, kidney function and inflammation in a salt-loaded rat model.

5) to examine if COX2 is involved in the vascular effects of lingonberry.

4 MATERIALS AND METHODS

4.1 Study models and designs

This project comprise of four studies. The studies are briefly presented with the main methods in Table 5.

Table 5. Study models and main methods.

	Study I	***Study II***	***Study III***	***Study IV***
Experimental model	SHR normal diet	SHR normal diet	SHR, WKY normal diet	WKY, normal and high-salt (HS) diet
Age (weeks)	6	6	9	8
n	6	6	8	8
Berry juice(s)	lingonberry, cranberry and blackcurrant	lingonberry, cranberry and blackcurrant	lingonberry	lingonberry
Treatment period	8 weeks	8 weeks	8 weeks 10 weeks (WKY)	10 weeks
Diet	Normal	Normal	Normal	Normal + 8 % NaCl
Methods	SBP, vascular reactivity		SBP, DBP, vascular reactivity	
Molecular biology methods		ELISA QPCR	ELISA Clinical chemistry Electrolytes ACE-activity	ELISA QPCR Clinical chemistry Electrolytes

4.1.1 Animal models (Study I-IV)

Spontaneously hypertensive rats (SHR) were used in studies I-III as a model of hypertension and vascular dysfunction. Hypertension in SHR is genetically programmed and starts to

develop when the animals are a few weeks old and increases to about 200 mmHg during their first two-three months (Pinto *et al.* 2018). Wistar Kyoto (WKY) rats were used as normotensive controls for SHRs (studies III-IV). Rats were purchased from Charles River laboratories (Sulzfeld, Germany) at the age of 5-6 weeks.

A high-salt diet was provided to the WKY rats in study IV as a model of diet-induced hypertension and inflammation. The high-salt diet was a normal rodent diet enriched with NaCl (8%). Control groups received a normal rodent diet (Harlan, Venray, The Netherlands).

Rats had free access to feed and drink. Rats were housed in a standard animal laboratory housed four to six in a cage according to their weight and baseline SBP so that each group (cage) had rats of approximately same mean weight and SBP.

The protocol of the studies was approved by the National Animal Experimentation Committee according to EC Directive 86/609/EEC and Finnish Experimental Act 62/2006.

4.1.2 Berry juices (Study I-IV)

Frozen berry purees were cold-compressed in the lab and diluted with water (1:3 in Studies I and II, 1:5 in Studies III and IV) every 3 days and stored in a fridge before being provided to the rats. Sucrose was added to the juice, in studies I-II, 4 % and in a more diluted juice (Studies III-IV), 1 %, to make the drinks more palatable. In studies III and IV, also the tap water given to the control groups was enriched with 1 % sugar. Drinking fluid and feed consumption were recorded by weighing the amounts before and after portioning to rats. The results were calculated per cage and divided by the number of rats in the cage. The polyphenolic contents of berry juices were measured by UHPLC and are listed in Table 6.

Table 6. Polyphenolic contents of the berry juices in studies I-IV assessed as mg/100g by UHPLC.

	Lingonberry	Blackcurrant	Cranberry	Lingonberry
Study	I, II	I, II	I, II	III, IV
Polyphenols total	65.2	55.4	29.4	34.1
Hydroxybenzoic acid	0.3	0.1	0.1	0.1
Hydroxycinnamic acid	5.3	4.5	5.2	2.5
Anthocyanins	14.4	39.9	12.4	5.9
Flavonols	11.0	6.5	5.7	3.8
Flavan-3-ols	11.2	nd	0.7	3.0
Procyanidins	23.0	4.4	5.3	18.7

4.2 Methods

4.2.1 Blood pressure measurement (Study I, III, IV)

Blood pressure was measured once a week using the tail-cuff method. In studies I and II, only SBP and heart rate were measured (Apollo-2AB Blood Pressure Analyzer, Model 179-2AB, IITC Life Science, Woodland Hills, CA, USA). In studies III and IV, DBP was also measured (CODA, Kent Scientific Corporation, Torrington, CT, USA). In each study, the measurements were made by the same researcher (Kivimäki) at the same time of day, before noon. Before the measurements, rats were warmed in a heated chamber (32 ˚C) for 10-20 min in aeriated cylinder tubes in order to make tail artery pulsation more intense and detectable. The measurement rooms were different; in studies III and IV, the blood pressure measurement was conducted in a more quiet and spacious room. In studies I and II, three correct and undisturbed consecutive SBP and HR measurements out of a total of ten were averaged. SBP, DBP and HR measurements in studies III and IV were conducted in three sets, each set consisting of eight measurements. Measurements accepted by the CODA-program were then calculated and averaged. Blood pressure measurement with CODA-instrument was more automated than with Apollo-analyzer.

Mean arterial pressure was calculated with the equation $\mathrm{DBP}+1/3\,(\mathrm{SBP}-\mathrm{DBP})$ in study III.

4.2.2 Vascular reactivity studies (Study I, III, IV)

At the end of the treatments, mesenteric arteries were dissected and cleaned from adherent connective tissue in pre-oxygenated Krebs-solution. From the proximal end of the mesenteric artery-aorta junction, a 3-4 mm section of a vessel was removed and the following 3-4 mm piece were used for reactivity studies. Mesenteric artery rings were hooked between stainless steel hooks and incubated in an organ-bath chamber for one hour with a resting tension of 1.0 g (Study I) and 1.5 g (Study III,IV) (volume 10 ml). Baseline levels of resting tension were taken into account when the force of constriction was calculated. Krebs solution in the chamber (pH 7.4–7.6, composition in mmol/l: NaCl, 119.0; $NaHCO_3$,

25.0; glucose, 11.1; $CaCl_2$, 1.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2) was pre-warmed in 37 ˚C and oxygenated with O_2/CO_2 (95%/5%, AGA, Riihimäki, Finland). An isometric force-displacement transducer (EMKA Technologies, Paris, France) was used for continuous measurement of the force of contraction by arterial rings.

After the incubation, the reactivity of the mesenteric arteries was tested by vasoconstricting or vasodilating drugs. Drugs were dosed to organ-bath chamber cumulatively (Ach, SNP) or once (PE, diclofenac, L-NAME).

Drugs and their mechanisms were as follows:

- Testing of contraction and relaxation ability of the arteries: 60 mM potassium chloride (KCl), 1 µM acetylcholine (Ach)
- Pre-contraction before endothelium- and non-endothelium-dependent relaxation: 1 µM phenylephrine (PE)
- Cumulative endothelium dependent relaxation: 1 nM - 0.1 µM Ach
- Inhibition of COX-derived relaxation: + 3 µM diclofenac
- Inhibition of eNOS-derived relaxation: + 100 µM L-NG-nitroarginine methyl ester (L-NAME)
- The role of EDHF: diclofenac + L-NAME
- Calcium-activated potassium channels K_{Ca} response: 0.1 µM apamin + 1 µM TRAM-34
- Cumulative endothelium-independent relaxation: sodium nitroprusside (SNP), 1 nM–0.1 µM

4.2.3 Biochemistry and clinical chemistry (Studies II-IV)

Collection of samples

Organ and blood samples were collected after the end of the experiment (study II-IV). Urine samples were collected during the 24 h metabolite caging (study III, IV) during treatment week 7.

Biochemical measurements

Biochemical measurements are summarized in Table 7. Enzyme-linked immunosorbent assays (ELISA) in studies II-IV are listed in Table 7 were conducted according to the manufacturer's instructions from plasma, serum or urine samples. Gene-expression analysis

assessed by the real-time quantitative polymerase chain reaction (RT-qPCR) (Study II, IV) were made from aortic samples. Prior to qPCR mRNA was isolated by Trizol Reagent (Invitrogen, Paisley, Scotland, UK) according to the manufacturer´s instructions. cDNA synthesis was performed from 1 µg of RNA by reverse transcription with SuperScript VILO cDNA Synthesis Kit (Life Technologies, Paisley, Scotland) according to the manufacturer's instructions. Primers for qPCR were designed with the National Center for Biotechnology Information (NCBI)/Primer-BLAST –tool. The primers were optimized to obtain 90-110 % efficacy. SYBR Green –chemistry reagents (Brilliant II SYBR Green master mix, Agilent Technologies) were used for relative quantitation. Relative gene-expression results were calculated using $2^{-\Delta\Delta Ct}$-method (Livak and Schmittgen 2001). ACE1 activity of serum and kidney lysate were measured according to Santos *et al*. (1985) (Study III, IV).

Table 7. Biochemical measurements in Studies II-IV. Plasma (p), serum (s), urine (u), aorta (a) or kidney (k) samples.

Biochemical variable	*Method*	*Sample*	*Study*
Aldosterone	ELISA	p	III, IV
Angiotensin II	ELISA	p	II
ADMA	ELISA	p	II
hs CRP	ELISA	p	II, III, IV
ICAM	ELISA	s	III, IV
IL-6	ELISA	p	II
IL-10	ELISA	p	III, IV
TNF α	ELISA	p	II
NOx	ELISA	p	II, III, IV
8-isoprostane	ELISA	p	II
6-keto PGF1α	ELISA	p	III, IV
Albumin	ELISA (urine)	u	III, IV
cGMP	ELISA (urine)	u	III, IV
Creatinine	ELISA (urine)	u	III, IV
ACE1, *activity	RT qPCR, *act. assay	a, s*, k*	II, III, IV
Adiponectin	RT qPCR	a	II
COX2	RT qPCR	a	II, IV
MCP1	RT qPCR	a	II
p-selectin	RT qPCR	a	II
V-CAM	RT qPCR	a	II

See list of abbreviations in page 7.

Clinical chemistry

Clinical chemistry measurements were conducted with automated ADVIA 1650 Chemistry System (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). Alanine aminotransferase (ALAT), alkaline phosphatase (ALP), aspartate transaminase (AST), creatinine (Crea), albumin (Alb), urea, creatine kinase (CKNAC), total bilirubin (TBil), inorganic phosphate (Pi), glucose (Glu), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL),

triglycerides (TG), total cholesterol (TC) and electrolytes (K, Na, Cl, Ca) were measured (study III, IV). Urine electrolytes and albumin were also measured in study IV.

4.2.4 Immunohistochemistry (additional data)

COX2 was measured immunohistochemically from kidney samples (Studies III and IV). Immunohistochemical analyses were made from paraffin-embedded 5 mm kidney sections. Paraffin blocks were sliced and fixed in Menzel-Gläser SuperFrost Ultra Plus -glasses. Paraffin was removed with Xylene-EtOH-H_2O washing protocol. Unmasking the epitopes was done in prior to actual staining with LabVision Autostainer (Thermo Fisher Scientific). The primary antibody was COX2 (D5H5) XP® Rabbit mAb (Cell Signaling Tehn.). Polyclonal HRP-Anti-Mouse IgG (ImmunoLogic, Netherlands) was used as a secondary antibody.

Images were generated using 3DHISTECH Pannoramic 250 FLASH II digital slide scanner at Genome Biology Unit supported by HiLIFE and the Faculty of Medicine, University of Helsinki, and Biocenter Finland. Pictures were analyzed with Case Viewer 2.3.

COX2 expression in the *macula densa* of kidney cortex: Randomly picked 40 glomeruli were calculated and the COX2 positive *macula densa* area was counted (%).

4.2.5 Molecular docking (additional data)

As described in Chapter 2.6.2, lingonberry contains kaempferol glycosides. Different 3D-structures where kaempferols (Preferred IUPAC name 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) are attached to proteins were searched from the Protein Data Bank (PDB) -database (www.rcsb.org). The orientation of kaempferol ring structures were analysed. Four of the 10 different structures in natural were selected and differences in rotation were evaluated.

The structure of a selective COX2 inhibitor, rofecoxib, was used as a model for kampferol binding to COX2. Structure (2.7 Å) of COX2 and rofecoxib, determined by X-ray crystallography, was used for modelling the kaempferol binding to COX2. The structure of COX2-enzyme (5KIR) was picked from the PDB (Orlando and Malkowski 2016). In this structure, rofecoxib is bound to active site of the COX2-enzyme. Kaempferol was fitted to active site according to ring structures of the molecules). The DeepView – Swiss-PDB-viewer (Swiss Institute of Bioinformatics, Lausanne, Switzerland) was used for docking and visualization of the structures.

4.2.6 Data analyses

Statistical data analysis was performed with IBM SPSS Statistics 20 or GraphPad Prism (Version 8) software using statistically appropriate tests suitable for this kind of analysis. T-test was used when only two groups were compared and ANOVA when four groups were compared. In study I, analysis of blood pressure and vascular function was conducted with ANOVA for repeated measurements. In studies III and IV, a general linear model for repeated measurements was used in the analysis of blood pressure and vascular function. A general linear model for univariate analysis was used to detect group differences in clinical chemistry measurements in studies III and IV. P value was considered significant when $p<0.05$. Data presented is mean ± standard error of mean (SEM) unless stated otherwise.

5 RESULTS

5.1 Weight gain, food and fluid consumption (Studies I-IV)

At the end of the treatment period, weight gain and relative organ weights (kidney, heart and left ventricle) were similar in all SHR groups (Study I-III). In study IV, high-salt (HS) groups had approximately 12 % lower body weights probably due to the unpleasant taste of the food and its smaller consumption and higher relative organ weights (% per body weight) of the kidneys, heart and left ventricle than rats with normal diet (Table 8).

Table 8. Relative organ sizes of the rats in study IV.

	Kidney %/body weight	Heart %/body weight	Left ventricle %/body weight
Control	0.31 ± 0.0	0.25 ± 0.0	0.21 ± 0.0
Lingonberry	0.31 ± 0.0	0.26 ± 0.0	0.21 ± 0.0
HS	0.41 ± 0.0***	0.30 ± 0.0***	0.23 ± 0.0***
HS + Lingonberry	0.40 ± 0.0***	0.30 ± 0.0***	0.24 ± 0.0***

***p<0.001 when compared to the control, ANOVA.

No adverse effects or abnormal behavior were seen in the rats. In studies I-II, rats drank less water than the berry juices. In study III, no significant differences were detected. In study IV, slight differences were seen in the consumption of the drinking fluid. The daily drinking amounts of water, lingonberry, HS, HS + lingonberry were as follows: 47 ± 4.2, 33 ± 3.5, 55 ± 4.9 and 42 ± 3.5 ml, respectively. The difference was significant only between rats with the HS *vs* lingonberry groups. The average feed intake was similar in all groups (from 18 to 20 g/d).

5.2 Polyphenol intake (Studies I-IV)

The daily intake of polyphenols per rat (mg/kg/day) is presented in Table 9. The intake of polyphenols was highest in the lingonberry group in studies I and II, when compared to cranberry and blackcurrant groups. In lingonberry juices, procyanidins were the most dominant polyphenol group, whereas in blackcurrant and cranberry groups, anthocyanins were the most abundant polyphenols.

Table 9. Polyphenol intake (mg/kg body weight) at treatment week 8.

	Lingonberry mg/kg	Blackcurrant mg/kg	Cranberry mg/kg	Lingonberry mg/kg	Lingonberry mg/kg	
Study	I, II	I, II	I, II	III	IV	
Polyphenols total	101.1	93.0	62.4	40.2	32.6[a]	44.6[b]
Hydroxybenz. acid	0.5	0.2	0.2	0.1	0.1	0.1
Hydroxycinn. acid	8.2	7.6	11.0	2.9	2.4	3.3
Anthocyanins	22.3	67.0	26.3	7.0	5.6	7.7
Flavonols	17.0	10.9	12.1	4.4	3.6	5.0
Flavan-3-ols	17.4	0.0	1.5	3.5	2.9	3.9
Procyanidins	35.6	7.4	11.3	22.2	17.9	24.5

a Normal diet, b HS diet.

5.3 Blood pressure (Studies I, III and IV)

Systolic blood pressure was significantly decreased during the eight weeks' treatment when compared to the control group in the older (9-17 weeks) rats (Study III) (Figure 5). The reduction of blood pressure was also reflected in the lower levels of mean arterial pressure (MAP) when compared to the control group (Figure 5). In the younger rats (6-14 weeks) investigated in Study I, provision of lingonberry did not ameliorate the rapid increase in blood pressure typically encountered in young SHR. In Study IV, blood pressure was not affected by lingonberry juice during the experiment.

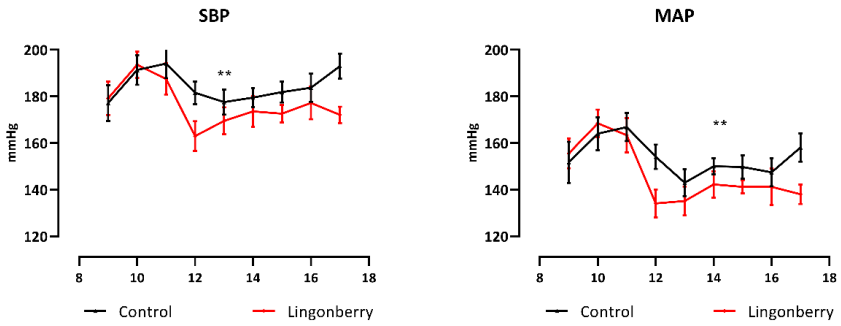


Figure 5. Systolic blood pressure (SBP) and mean arterial pressure (MAP) of hypertensive rats in study III.** p<0.01, General linear model for repeated measurements.

5.4 Vascular reactivity (Studies I, III and IV)

In study I, the maximal acetylcholine-induced relaxation of mesenteric arteries was 75 % of the pre-existing contraction in the control group of SHR after the eight-week study period. In the lingonberry group, the response to acetylcholine was enhanced by as much as 100 % (Figure 6). Diclofenac (a COX inhibitor) had no effect on the extent of the relaxation, whereas L-NAME (NOS inhibitor) abolished the Ach-induced relaxations completely in the control SHR group but only partly in the lingonberry group. This residual relaxation in the presence of L-NAME and diclofenac in the lingonberry group was almost totally abolished by apamin (small conductance K_{Ca}, SK_{Ca} inhibitor) and TRAM (intermediate conductance KCa, SKCa inhibitor). Cranberry and blackcurrant treatments did not show any significant improvements in the relaxation of the vessel. The endothelium-dependent relaxations from Study I are illustrated in Figure 6.

In the mesenteric arteries of SHR examined in study III, no significant improvements were seen in the acetylcholine response after lingonberry treatment. Consumption of a high-salt diet did not affect the vascular response (Study IV).

Sodium nitroprusside, a NO donor, induced an endothelium-independent relaxation which was enhanced in the lingonberry group when compared to the control group in Study I (Figure 6). In study III, endothelium-independent relaxation did not significantly differ between the study groups indicating that the relaxation effect of lingonberry juice on mesenteric arteries of SHR was endothelium-dependent.

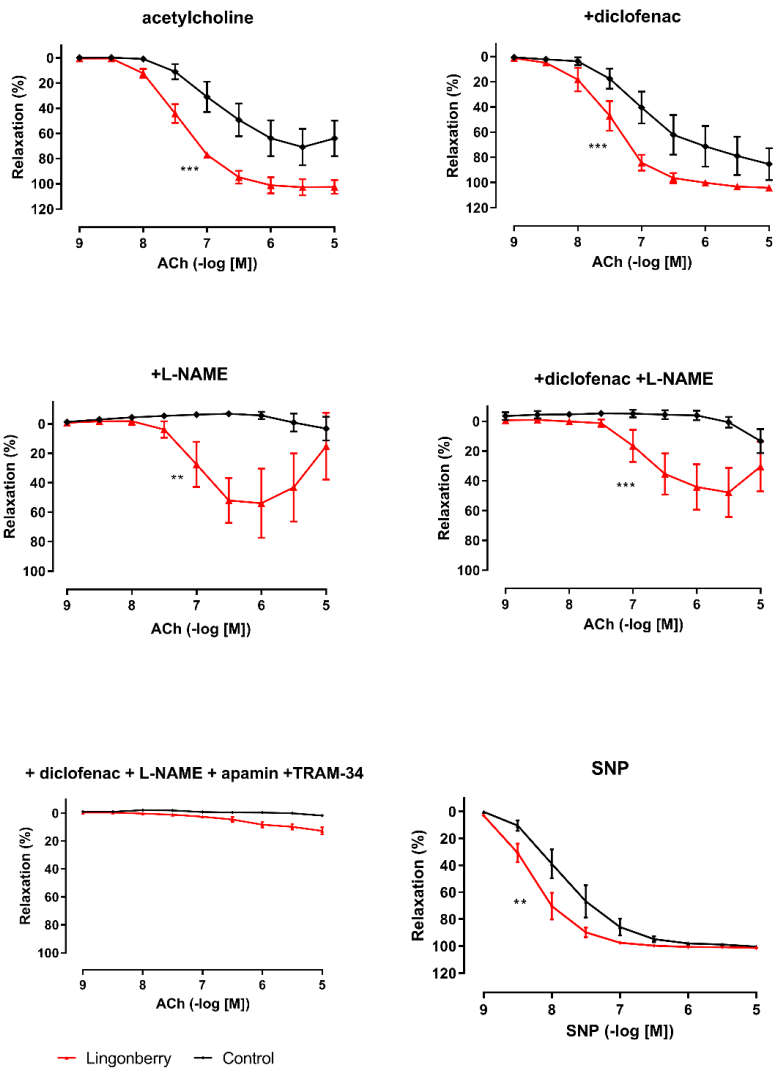


Figure 6. Endothelium-dependent (Ach) and –independent (SNP) vascular responses of mesenteric artery rings after the eight-week treatment in study I. *p<0.05, ** p<0.01, ***p<0.001, ANOVA for repeated measurements.

5.5 Renin-angiotensin system and nitric oxide (Studies II-IV)

The effects of long-term treatments on renin-angiotensin system were evaluated by RAS-markers from serum/plasma, kidney and aortic samples (Table 10).

Table 10. The effects of berries on markers related to the renin-angiotensin system.

	Study	Sample/method	Results	
Angiotensin II	II	Plasma/ELISA	Lingonberry	↓*
			Blackcurrant	↓*
ACE1	III, IV	Plasma/ELISA		↔
	II	Aorta/mRNA	Lingonberry	↓*
			Cranberry	↓*
ACE1 activity	III	Serum		
		Kidney		↔
Aldosterone	III, IV	Plasma/ELISA		↔
Total NO	II, III, IV	Plasma/ELISA		↔

*p<0.05, ANOVA (II, IV) or General linear model for univariate analysis (Study III).

The concentrations of angiotensin II peptide in plasma samples were reduced by lingonberry and blackcurrant treatments in study II. In the same experiment, lingonberry treatment down-regulated ACE1 gene expression in the aorta, also cranberry treatment reduced the expression. Serum and kidney extracts were used to determine ACE1 activity in study III, no differences were observed. Furthermore, in study IV, no differences were found between the groups in their plasma ACE1 protein levels.

The aldosterone level in plasma was not affected by the treatments in studies III and IV. Furthermore, total NO was unchanged in studies II-III.

5.6 Inflammation and COX2 (Studies II, III and IV)

5.6.1 Clinical chemistry

Clinical chemistry analyses were conducted to evaluate the effects of the treatments to the basic functions and electrolyte balance in rats.

Serum alkaline phosphatase levels were reduced by lingonberry treatment in hypertensive rats (Figure 7). The tendency to similar direction was also seen in the normotensive WKY rats with or without high-salt diet.

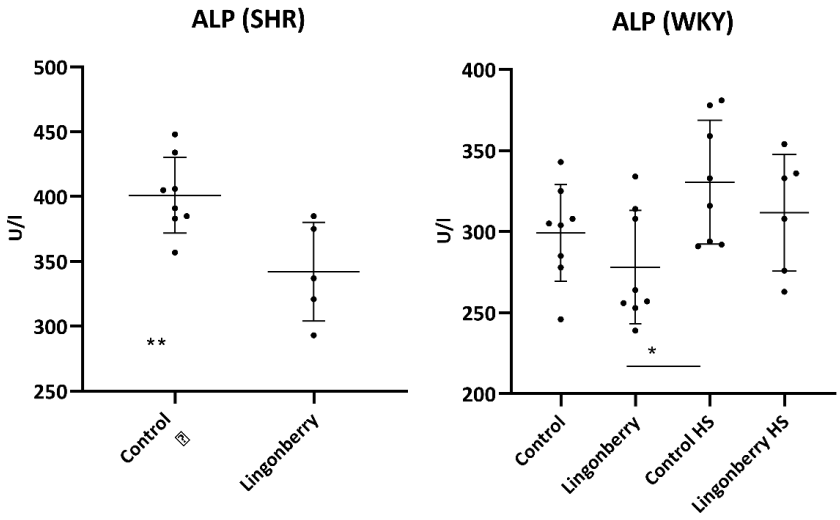


Figure 7. Serum alkaline phosphatase (ALP) in hypertensive (SHR) and normotensive rats (WKY) with or without salt loading (HS) after the eight-week treatment period. p<0.05, **p<0.01, t-test (SHR), General linear model for univariate analysis (WKY).

Lingonberry treatment increased serum calcium and chloride concentrations when compared to the control group (Table 11).

Table 11. Clinical chemistry data of serum samples from studies III and IV.

		Study III		Study IV			
		Control	Lingonberry	Control	Lingonberry	HS	HS Lingonb.
Ca	µmol/l	2.8 ± 0.0**	3.0 ± 0.1	2.8 ± 0.0	2.8 ± 0.0	2.7 ± 0.0	2.7 ± 0.1
Cl	µmol/l	104 ± 0.4*	105 ± 0.4	105 ± 0.5	103 ± 0.5	106. ± 0.8	108 ± 0.3
Chol	mmol/l	2.2 ± 0.1	2.4 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.2
HDL	mmol/l	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
LDL	mmol/l	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
TG	mmol/l	1.3 ± 0.1	1.1 ± 0.1	2.0 ± 0.2	2.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
Alb	g/l	41.7 ± 0	41.4 ± 1	38.5 ± 0	38.4 ± 0	37.5 ± 1	36.5 ± 1
Urea	mmol/l	7.2 ± 0.3	7.3 ± 0.2	7.4 ± 0.1	6.8 ± 0.01	9.4 ± 0.12	9.4 ± 0.1

**p<0.01, *p0.05, t-test (Study III), ANOVA for univariate analysis (Study IV).

5.6.2 Inflammatory variables and effects of salt-loading

As expected, HS diet induced a slight increase in inflammatory markers. Cyclic GMP, 8-isoprostane and albumin levels in urine were increased (Table 12.) (Study IV). In the lingonberry group, levels were slightly lower, but differences were not statistically significant. Inflammation and kidney stress were seen in increased relative kidney, heart and left ventricle weights in salt-loaded rats (presented in Chapter 5.1). Interestingly, ICAM was lowered by consumption of a high-salt diet and even more with lingonberry treatment when compared to the control group. In SHR (Study III), no differences were seen in variables measured from urine (cyclic GMP, 8-isoprostane and albumin).

The effects of salt-intake were seen also in increased urine concentrations of sodium and chloride (Table 12) and the excretion of calcium was also increased.

Table 12. Inflammatory variables in the urine. Values (± SD) are set in proportion to mg creatinine.
*p<0.05, ** p<0.01, ***p<0.001 when compared to the control group, ANOVA for univar. analysis.

		Control	Lingonberry	HS	HS Lingonberry
8-isoprostane	pg/mg	5.4 ± 1.1	4.4 ± 1.3	9.3 ± 2.4*	8.0 ± 2.7
cGMP	pmol/mg	0.45 ± 0.1	0.71 ± 0.3	1.58 ± 0.2**	1.68 ± 0.3**
Albumin	ng/mg	234 ± 25	346 ± 39	1216 ± 240***	1215 ± 223***

5.6.3 Gene expressions

Lingonberry juice affected the gene expressions of inflammatory and atherothrombotic markers (Table 13). The levels of MCP1 cytokine was reduced in lingonberry group in study II. Relative gene expression analysis showed that there was about a 50 % reduction in MCP1 gene expression after lingonberry treatment. The relative expression of p-selectin was also significantly lowered in lingonberry and cranberry groups. In addition, VCAM-1 expression was lower in the lingonberry group. In study IV, gene expressions of VCAM-1 and p-selectin did not differ between the study groups. COX2 gene expression was significantly down-regulated by lingonberry treatment in study II. Blackcurrant juice decreased the expression, but the effect was less impressive than that achieved with lingonberry. In study IV, COX2 relative gene expression was increased by about 50 % after the consumption of the HS diet when compared to control group. Lingonberry treatment lowered the expression in SHR to the same level as that observed in WKY.

Table 13. Inflammatory and atherothrombotic markers. Relative gene expressions in the aorta in Study II.

	COX2	MCP1	P-selectin	VCAM-1
Cranberry	↔	↔	↓**	↔
Lingonberry	↓**	↓***	↓***	↓***
Blackcurrant	↔	↔	↔	↔

↔=ns, **<0.01, ***<0.001, ANOVA.

5.6.4 COX2 and the kidneys (additional data)

Strong signals of COX2 were seen in the *macula densa* area in the kidney cortex with immunohistochemical staining. COX2-positive *macula densa* area in the kidney cortex was significantly increased in lingonberry-treated hypertensive rats. The HS diet inhibited the COX2 expression in the *macula densa* area (Figure 8) (Study IV). The expression was highest in the lingonberry treated rats consuming the normal food.

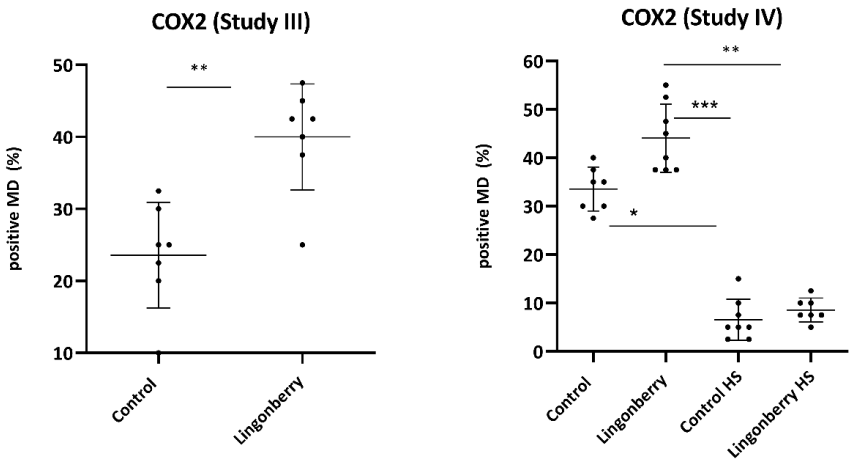


Figure 8. Positive *macula densa* area in the kidney cortex of the rats. *=p<0.05, **=p<0.01, p<0.001, t-test (Study III) or General linear model for univariate analysis (Study IV).

5.6.5 Molecular docking, COX2

Differences between kaempferol orientation in complex structures bound from PDB (proteins pdb-code: 4det, 5aux, 2c1z, 3qwh, 4el9, 4rel, 5av2, 5av3, 1h1m ja 6m8b) is illustrated in Figure 9. Torsion angle between ring structures does not differ much between different structural orientations.

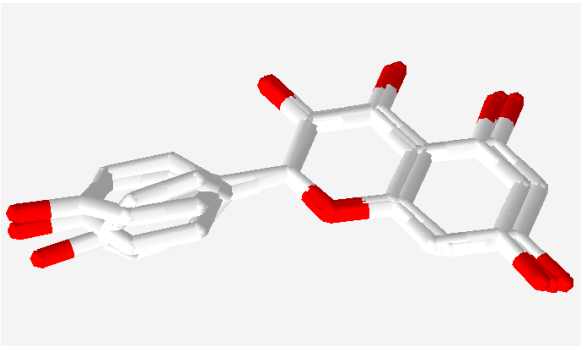


Figure 9. Kaempferol rotations. White is carbon, red is oxygen.

According to bounding model of rofecoxib, kaempferol could be bound to COX2 by four different orientations (Figure 10A-D). Kaempferol has the ability to similar binding as rofecoxib due to binding interactions and steric effects.

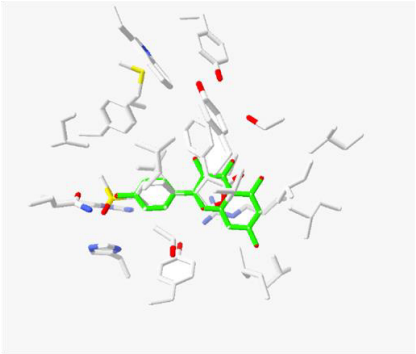


Figure 10A. Kaempferol in the active site of COX2, mod1.

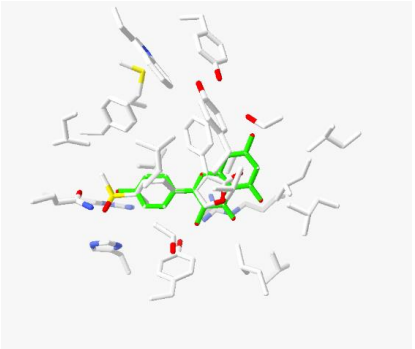


Figure 10B Kaempferol in the active site of COX2, mod2.

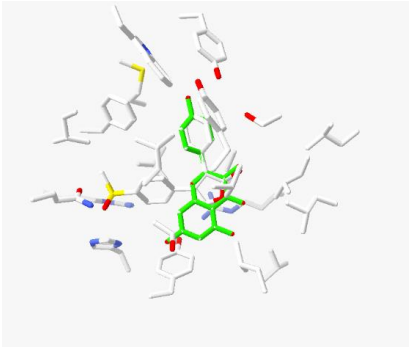


Figure 10C. Kaempferol in the active site of COX2, mod3.

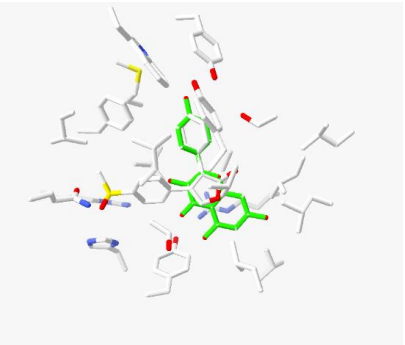


Figure 10D. Kaempferol in the active site of COX2,mod4.

In the molecular structures in Figures 10 A-D, white is carbon (C), red is oxygen (O), nitrogen (N) is blue and yellow is sulphur (S).

6 DISCUSSION

In this series of four studies, three berries and their effects on blood pressure, vascular function and inflammation were investigated in experimental studies with hypertensive and normotensive rats. Lingonberry, cranberry and blackcurrant juices were compared in the first two studies and lingonberry juice was chosen for the further two studies.

Lingonberry juice as drinking fluid was shown to enhance vascular function and reduce systolic blood pressure and mean arterial pressure. It was not possible to observe any dose-dependency for these vascular effects in this series of studies. Endothelium-dependent vasodilatation was found to be mediated via nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). In addition, lingonberry has displayed anti-inflammatory and anti-atherothrombotic effects.

Antihypertensive effects of lingonberry

The systolic blood pressure of spontaneously hypertensive rats was lower in the lingonberry treated rats during the eight-week study period (III). Lingonberry juice also attenuated the age-related elevation of the systolic blood pressure encountered in the last weeks of the study period. Mean arterial pressure was lower in rats treated with lingonberry juice at the end of the study. As far as is known, this was the first study where the blood pressure lowering effect of lingonberry consumption has been demonstrated in experimental animals. However, there are clinical studies revealing the blood pressure lowering effects of other polyphenol rich berries, like blueberry and cranberry (Johnson *et al.* 2015; Novotny *et al.* 2015) and studies conducted with berry mixes including also lingonberry (Erlund *et al.* 2008). In the first study, where a few weeks' younger SHR rats were used, lingonberry juice was not able to abolish the blood pressure increase typical for SHRs. Antihypertensive effects were not detected after consumption of cranberry or blackcurrant juices. Interestingly, consumption of a high-salt diet did not markedly increase the blood pressure of normotensive WKY rats and possibly therefore no significant antihypertensive effect of lingonberry juice was evident (IV).

The age of the rats exerts an influence on blood pressure development and possibly on the effectiveness of berry treatments. The rats were three weeks younger in the first study, and in these young spontaneously hypertensive rats, lingonberry juice was not able to attenuate the strong genetically-mediated increase in blood pressure (preventive effect) (Pinto *et al.* 1998). However, when the blood pressure had already stabilized at a high level, lingonberry juice treatment decreased the systolic blood pressure and was also able to attenuate the increase in the last weeks of the study, when SHRs were anticipated to display maximal SBP

values (over 180 mmHg). The concentration of lingonberry juices differed between the experiments, because of the changes in growing conditions and also different dilutions were used. In the third study, the effect of dose on blood pressure and vascular function were tested. The blood pressure lowering effect was more evident with the more diluted juice, containing about half of the polyphenol content when compared to the stronger juice administered in the first study. Quinones *et al*. (2011) made a similar observation when a cocoa polyphenol extract was administered to SHR, i.e. the two times more concentrated extract did not decrease the blood pressure, in contrast to the more diluted version. Phenolic compounds are usually anti-oxidative, but paradoxically, they might become pro-oxidative and produce ROS (Castaneda-Arriaga *et al. 2018).* The pro-oxidative effect is dose-dependent and influenced by environmental conditions, e.g. pH and the presence of transition metal concentrations can affect the reactions. This fact can support our findings. In addition to the pro-oxidative theory, the different blood pressure measurement methods utilized in Studies I and III might, at least partly, explain this phenomenon.

The effects on vascular function

The effects of lingonberry juice on vascular function were seen when vascular responses were assessed after the treatment periods. In lingonberry juice-treated rats, the impaired endothelium-dependent relaxation typical for SHR was totally normalized. No significant improvement in vascular reactivity was seen in a few week older rats after eight weeks' treatment. The endothelium-dependent relaxation in study I was not totally abolished by inhibition of either COX or NOS, indicating that in addition to NO, also EDHF contributed to the endothelium-dependent relaxation of lingonberry-treated rats. In smaller arteries, including mesenteric artery, NO-independent relaxation has a more dominant role than in major arteries, such as aorta (Mulvany and Aalkjaer 1990). Experimental studies with other polyphenol-rich products, such as red wine, blackcurrant, berry juices (Auger *et al.* 2011; Auger *et al.* 2015; Rashid *et al.* 2018) and resveratrol (Li *et al.* 2016; Fabricio *et al.* 2017) have detected enhanced endothelium-dependent relaxations. Tabart *et al*. (2018) noted that in commercial blackcurrant juices, the extent of the relaxation of porcine coronary arteries correlated with the total anthocyanin content. This could partly explain the differing results between studies I and III, since the higher polyphenols and anthocyanin content in study I apparently affected the relaxation.

Mechanisms of action

The important blood pressure regulator, the renin-angiotensin system, was affected by berry treatments. Lingonberry consumption lowered the plasma level of angiotensin II, also gene expression of the Ang II producing enzyme ACE1 was diminished in the aorta after eight weeks' treatment. Together with lingonberry, blackcurrant lowered circulating Ang II and cranberry lowered ACE1 gene expression in the aorta. However, no changes in circulating ACE1 protein were seen after berry treatments.

Angiotensin II is a powerful vasoconstrictor (Khanna *et al.* 2017) but it also acts to induce inflammation and is active in several other pathological states (Montezano *et al.* 2014). ACE1 inhibition *in vitro* has been shown previously with polyphenol-rich cocoa, tea and red wine (Actis-Goretta *et al.* 2006), also blueberries have lowered circulating ACE levels in rats (Wiseman *et al.* 2011). Apparently, at least flavanols, flavonols, flavan-3-ols and procyanidins have an ability to inhibit ACE1 *in vitro* (Actis-Goretta *et al.* 2003, 2006). This is in line with our data from study I, where the intake of flavan-3-ols and procyanidins was highest in lingonberry treated rats. The lower circulating level of Ang II is in line with lowered gene expression of ACE1, since Ang II is formed by cleavage from angiotensinogen by ACE1. The inhibition of Ang II expression by resveratrol was reported in aging kidney of the mice together with the reduced expression of ACE1 (Jang *et al.* 2018). The reduced level of Ang II in the circulation might also be related to the inhibition of inflammation.

The aortic relative gene expressions of COX2, MCP1, P-selectin and VCAM1 were down-regulated after lingonberry treatment. The results of gene expression data support the theory that lingonberry juice has anti-inflammatory properties involving a possible role of the NF-kB pathway, as previously has been described for polyphenols (Hämäläinen *et al.* 2007; Rius *et al.* 2010). The down-regulation of p-selectin and VCAM1 is related to anti-thrombotic actions and endothelial function (Mozos *et al.* 2017). Circulating concentrations of VCAM1, LDL and total cholesterol levels have been shown to be lowered after 8 weeks' consumption of strawberries in a clinical study with overweight subjects (Basu *et al.* 2010). At the same time, NO has been shown to affect inflammatory processes *e.g.* inhibiting the effects of adhesion molecules and MCP1 (Takahashi *et al.* 1996; Ewart *et al.* 2008).

The synthesis of prostaglandins and thromboxane A2 together with other prostanoids is mediated by COX. Overexpression and increased activity of COX2 have been shown in small resistance arteries of hypertensive patients impairing the availability of NO (Virdis *et al.* 2013). Thus, inhibition of COX2 expression in aorta could partly enhance the bioavailability of NO. In summary, these possible anti-inflammatory actions of lingonberry could be due to an enhancement of the production and bioavailability of NO. This is also supported by the finding of endothelium-dependent relaxation, which was partly mediated by NO. Previously, endothelial NO production was found to be enhanced by consumption of a polyphenol-rich diet in subjects with a high cardiovascular risk (Medina-Remon *et al.* 2015).

Serum alkaline phosphatase was markedly reduced by lingonberry treatments in studies III and IV. Circulating ALP is considered to be an independent and strong predictor of all-cause

mortality in patients with chronic kidney disease as well as in the general population (Haarhaus *et al.* 2017). Serum ALP activity has been associated with CRP and inflammation (Nayeem *et al.* 2010; Damera *et al.* 2011; Seo *et al.* 2019). Interestingly, endothelial dysfunction (Perticone *et al.* 2015) and the phenomenon of a slow coronary flow (Wang *et al.* 2018) also seem to be related to the increased ALP levels.

Interestingly, the expression of COX2 protein in the *macula densa* area of the kidneys was significantly increased in lingonberry juice-treated hypertensive rats and a minor increase was seen also in normotensive rats. In contrast, the high-salt diet almost completely abolished the expression. As discussed earlier, COX2 has a dual role and especially in the kidneys, it is constitutively expressed and contributes to the regulation of blood flow. In the kidneys, COX2 is regulated by Ang II and AT1 and AT2 receptors (Zhang *et al.* 2006). In cortex and *macula densa*, COX2 mediates sodium balance and the regulation of renin release (Harris and Breyer 2001). Two RAS components, Ang II and aldosterone, decrease COX2 expression in the *macula densa* area (Cheng *et al.* 1999) and Wolf et al. (1999) revealed that the inhibition of Ang II upregulated COX2 expression in the *macula densa* and the effect is mediated via AT1 receptors. The COX2 expression in *macula densa* is increased during salt deprivation (Harris *et al.* 1994). Cheng *et al*. (2000) found that in addition to low-salt, captopril, an ACE1 inhibitor, strongly induced COX2 expression in Sprague-Dawley rats. When compared to previous results, the increased expression of COX2 protein in *macula densa* by lingonberry could be a reflection of inhibition of RAS, especially ACE1. As far as is known, this is the first time when polyphenol-rich foods have been shown to increase COX2 expression in the *macula densa* area.

A high-salt diet impaired the kidney function of young rats, whereas blood pressure or vascular function were not affected. The increased sizes of the kidneys, heart and left ventricle together with increased urinary albumin, cGMP and 8-isoprostane can be regarded as reflections of the presence of the oxidative stress induced by high-salt diet.

Polyphenols in action and safety issues

The effects of polyphenols are apparently dependent on the type and dose of the polyphenol(s) being administered. Some plateau- or saturation point seems to exist. For instance, acute flow-mediated dilatation (FMD) was enhanced dose-dependently by procyanidins until a dose of 0.5 g/d; with catechol flavonoids, the corresponding amount was 0.2 mg/d (Kay *et al.* 2012). Metabolism is affected by dietary factors and it seems to be that too high a dose might decrease the bioavailability, as has been seen with chlorogenic acid (Stalmach *et al.* 2014). A critical point in polyphenol or berry studies is to provide a suitable control product. The actions of polyphenols are affected by their chemical nature and bioavailability, which differs greatly between phenolic compounds. As discussed earlier, the bioavailability is a result of several mechanisms which are affected by the food matrix, intestinal microbes and other factors. The health-promoting effects are partly due to the

polyphenols themselves and partly due to their metabolites. Polyphenols are secondary metabolites of the plants, their concentration is affected by growing conditions, such as UV-light, dryness and pathogens. Thus, the polyphenolic content may vary between growing site and years due to different growing circumstances (Bujor *et al.* 2018). In addition to the concentration differences in our studies, there were slight differences in the polyphenolic composition of the juices. The more diluted juice contained a relatively higher amount of procyanidins, but the intake of procyanidins was higher from the more concentrated juice. There might also be some undetected differences in the polyphenolic compositions of the juices, due to different growing places and harvesting years. We were not able to detect these kinds of differences with the methods available.

In the molecular docking model, we showed that the kaempherol structure could theoretically bind and act as inhibitor of COX2. Kaempherol was modelled to active site of COX2 using rofecoxib, a selective non-steroidal anti-inflammatory drug, as a template. Lingonberry contains several different kaempherols (Bujor *et al.* 2018). Since the torsion angle changes of different kaempherols did not differ to any great extent, several potential inhibitor structures are possible.

Berries are considered as safe to use. Caution is needed only when pure polyphenols are used. Pure polyphenols or high amounts of polyphenol-rich foods can interact with some medicines metabolized through CYP-family (Basheer and Kerem 2015). In those cases, the effect of the drug can be either inhibited or enhanced. In this series of experiments, relatively high amounts of polyphenols were ingested in berry drinks. No adverse effects were found. The accepted daily intake for lingonberry polyphenols is not established. The EFSA has stated that as a food ingredient, anthocyanin can be used with a *quantum satis* principle. The LD50 value has been determined for an extract containing cyanidin, petunidin and delphinidin, for mice, the LD50 value was 25 g/kg; for rats, it was 20 g/kg (Wallace and Giusti 2015). In a review of anthocyanin RCTs, no adverse effects were reported after the consumption of anthocyanin concentrations up to 0.640 g/day (Wallace *et al.* 2016).

Methodological aspects

Spontaneously hypertensive rats are a widely used model for hypertension. Their systolic blood pressure increases to over 200 mmHg within a few months (3-4) after birth (Okamoto and Aoki 1963). Wistar Kyoto rats are the normotensive reference strain for SHR. In our last study, we wanted to increase the blood pressure by high-salt diet in order to reflect nutritional aspects behind the increase in blood pressure. Blood pressure did not increase during our 10 weeks' treatment, probably due to the fact that the salt exposure was too short and mineralocorticoid treatment was not added. However, we were able to observe other effects caused by high-salt diet.

Lingonberry juice was too astringent for rats and therefore sucrose was added to make it more palatable for the animals. Sucrose was added to the control animals´ drinking water in studies III and IV to avoid bias. Lingonberry treatment did not affect the weight gain of the rats (Studies I and III). Normotensive WKY rats consuming a high-salt diet were lighter than WKYs without salt.

Blood pressure was measured by the tail-cuff method, which is a widely used and well-known non-invasive method used in experimental studies. At first, rats were acquainted with the measuring technique, and they readily adapted to the procedure. The blood pressure assessments were made by the same researcher at the same time of day to minimize errors in the measurements. In study I, an older measurement device (Apollo) was used to monitor the signal from the cuff, whereas in Studies III and IV, newer equipment (CODA) was available. The measurement of blood pressure was more reliable and possibly more accurate with CODA, since more measurements could be made, and also diastolic blood pressure was more stable. In Study III, we also had the opportunity to use a more modern and quiet examination room for our blood pressure measurements, which possibly may have been less stressful to the animals. This might partly explain the differences in the effects on blood pressure.

Vascular responses were studied in an organ-bath chamber with an isolated superior mesenteric artery (resistant artery) of the rats. Mesenteric arteries were suitable for detecting vascular resistance and hypertension. Aortic sections were used for mRNA studies. Due to practical problems, resting tensions were adjusted to different levels in the studies. For example, a higher resting tension was more appropriate for larger rats and tension remained throughout the rather prolonged test period. Baseline resting tension was reduced from the force of contraction in order to balance the differences between samples. However, this might have a slight influence on the divergent results found in the different studies.

Clinical relevance and future prospects

Nutrition plays a major role in the prevention and treatment of hypertension and cardiovascular disease. Lifestyle guidance is recommended in Finnish Current Care Guidelines of Hypertension (2014). It is evident that even minor reductions in systolic and diastolic blood pressure can decrease an individual's risk of stroke, heart failure and coronary artery disease. It is possible to achieve a moderate reduction of blood pressure by consuming polyphenol-rich foods. Especially, in the Nordic countries, wild berries are the best source of bioactive polyphenols.

Results from these experimental studies cannot be extrapolated directly to clinical situations in humans. However, important pre-clinical mechanistical information and indication of actions were obtained and the results can be utilized in clinical trials.

These actions are related to the polyphenolic profile of the berry. In our study, cranberry juice did not affect vascular function, but there are some clinical indications for possible anti-inflammatory and anti-infective actions of cranberry, especially in conjunction with urinary tract infections (Foxman *et al.* 2015; Mantzorou and Giaginis 2018). Clinical studies with lingonberries will be needed to clarify its benefits for human health. Nevertheless, some of our preclinical results as well as the work of others are encouraging, i.e. lingonberry may be able to improve vascular function and to exert positive effects on cardiovascular health including inflammation and oxidative stress.

ACE1 inhibitors are primary drugs for the treatment of hypertension (Williams *et al.* 2018). In the USA, the classification and the recommendations for lowering the blood pressure are being re-evaluated (Whelton *et al.* 2018). This would be expected to cause an increased need for the use of medication. In cases of mild hypertension, ACE1 inhibiting polyphenol-rich foods and supplements could be used as a non-medical treatment with less adverse effects than pharmacological agents, or perhaps as a sensitizer of drug treatment. The dual role of COX (inducible and constitutive) leads to the fact that NSAIDs possess adverse effects especially on renal balance (Curtis *et al.* 2019). As was shown in this thesis, lingonberry is able to inhibit vascular, probably inducible, COX2 while still maintaining the expression of constitutive COX2 in the kidneys. This phenomenon should be investigated further and utilized as a safer treatment for inflammatory states.

This series of studies revealed that lingonberry juice has the potential to decrease blood pressure and improve vascular function in experimental models. However, possible dose dependency needs further clarification. The inhibition of the renin-angiotensin system, especially ACE1 inhibition and thus the ability to prevent the detrimental effects of angiotensin II together with enhancing NO bioavailability are potential mechanisms accounting for these positive vascular effects. These effects seem to be dependent on the concentration and the polyphenolic profile of the berries. Lingonberry juice treatment exerted anti-inflammatory actions, which most likely contributed to the improved vascular function. The property of lingonberry juice to inhibit inducible COX2 in vasculature together with the preservation of important constitutive renal COX2 are promising, but these putative mechanisms need to be confirmed with more detailed experimental evidence and clinical approval.

7 SUMMARY AND CONCLUSIONS

In this series of four studies, we investigated the effects of three berries, lingonberry, cranberry and blackcurrant on blood pressure and vascular function in experimental models of hypertension. A special focus was placed on lingonberry and its effects on the renin-angiotensin system, its anti-inflammatory actions; the role of COX2 was also evaluated. Here are the main findings emerging from this series of studies:

- Consumption of lingonberry juice lowered the already elevated blood pressure of spontaneously hypertensive rats. Lingonberry juice was not able to prevent the development of genetic hypertension in these rats and no dose-dependency was observed.

- The impaired endothelium-dependent relaxation of SHRs was normalized after lingonberry juice consumption in younger rats with a high dose, but no enhancement was seen in older rats receiving a lower dose. The higher dose of lingonberry juice also ameliorated endothelium-independent relaxation of mesenteric arteries.

- Lingonberry juice might reduce the extent of inflammation related to hypertension and vascular dysfunction. Its anti-inflammatory mechanism seems to be related to inhibition of certain proteins and peptides (COX2, Ang II, MCP1) and atherothrombotic (MCP1, p-selectin, VCAM) markers. The reduction of the circulating levels of alkaline phosphatase might also have a positive effect on endothelial function and vascular health.

- The blood pressure lowering effects of lingonberry juice are possibly related to the renin-angiotensin system i.e. its partial inhibition. Circulating levels of Ang II were reduced in plasma and the aortic gene expression of ACE1 was down-regulated. Lingonberry juice treatment increased the expression of COX2 in the *macula densa*, which also might contribute to the inhibition of RAS.

- A moderate abolishment of inflammation and renal damage induced by a high-salt diet and possible anti-inflammatory effects were seen, but more definitive conclusions will need further studies.

8 ACKNOWLEDGEMENTS

These studies were conducted in the Department of Pharmacology of the Faculty of Medicine, University of Helsinki during 2010-2019. This has been a long journey and I am grateful for this opportunity to complete this PhD thesis. I want to thank the former head of the department, Professor Esa Korpi and current leader Professor Eero Mervaala for providing such excellent facilities to do scientific work. This project has been funded by the Foundation for Nutrition Research, the Juhani Aho Foundation for Medical Research, Finska Läkaresällskapet: Einar och Karin Stroems Stiftelese, Business Finland, the Finnish Atherosclerosis Society and Doctoral School in Health Sciences.

I will be forever grateful to my magnificent supervisors, Professor Heikki Vapaatalo and Professor Riitta Korpela, for all the time and guidance given during this journey. The guidance that both of you provided has been excellent and I admire your knowledge and passion for science. Your doors have always been open. I want to express my deepest gratitude to Heikki for the boundless encouragement, inspiring discussions and patience to wait for me to return to scientific matters. Riitta, your support has always been motivating and you have always believed in me. Your positive attitude and lively discussions have given me the strength to proceed.

My inspiring thesis committee members Professor Marina Heinonen and Docent Matti Jauhiainen are acknowledged for the scientific support for this project. I want to express my gratitude to the official reviewers of this thesis, Docent Riitta Törrönen from University of Eastern Finland and Docent Pertti Arvola from Tampere University Hospital for their valuable comments and the major improvements made to this thesis. Dr. Ewen MacDonald is acknowledged for revising the language of this thesis.

I feel fortunate that I have had such a good team and collaborators when doing this work. My co-authors and dear friends, Dr. Pauliina Ehlers and Dr. Aino Siltari, thank you; it has been a pleasure to work with you and I have happy memories of the moments shared with you in personal life too. I want to thank also my co-author Docent Anu Turpeinen for motivating and seamless collaboration. Dr. Jarkko Valjakka from the University of Tampere is warmly acknowledged for teaching me the fundamentals of molecular docking. I am very excited that I had an opportunity to collaborate and learn from Professor Sanna Lehtonen and I also want to thank her research group for the help with kidney samples. I am grateful for the valuable scientific advice and stimulating discussions with Docent Piet Finckenberg during these years.

I want to thank warmly my friends and former group members Hanna Ventola, Liisa Lehtoranta, Lotta Stenman and Nora Jaatinen for the memorable moments and support in the ups and downs. My current group members, Richard Forsgård, Hanne Salmenkari, Tuomas Heini, Veera Kainulainen are sincerely acknowledged for the lively discussions, good times and immaculate work. It has been fun to work with you all!

It has been an excellent time to work in the Department of Pharmacology. I want to thank all of the former and current staff members for nice discussions and lively atmosphere. Anneli von Behr, Nada Bechada-Hirvonen and Lahja Eurajoki are warmly acknowledged for the help and guidance in laboratory activities and practical aspects. Päivi Leinikka, Sari Laakkonen and laboratory animal unit in Biomedicum are greatly acknowledged for their help with the experimental studies. Maarit and others in the instrument maintenance unit, thank you for your contributions in ensuring laboratory works. Thank you Anne Simola for helping with practicalities.

Docent Pirkko Pussinen is gratefully acknowledged for the guidance in the scientific world and also all nice moments outside the lab. I want to thank Susanna Paju, Kati Hyvärinen, Aino Salminen and Elisa Kallio for all memorable moments during these years. Milla Pietiäinen and Laura Lahdentausta, thank you for enjoyable company and encouragement during last parts of this project. Airi Sinkko and others, thank you for the nice times in the dental lab. Taina Tervahartiala is warmly acknowledged for the lingonberry pictures.

It is delightful to be surrounded by such nice people. Thank you Marjo for being a fantastic friend, since we were teenagers, we have shared the ups and downs. Maria, my cousin and dear friend, thank you for your support and understanding in every aspects of life which means so much to me. You both are very dear to me. Thank you Elina, Iida and Lauri, for all enjoyable moments and our long-lasting friendship. Our lovely neighbours Anita and Miika are greatly acknowledged for nice company and help with the children. Without friends this journey would have been much longer.

My parents, Reetta and Antti, thank you for your endless support during my all life and the knowledge that I can always rely on your help. Without your help with the child care, this book would never be published. My very wonderful sisters, Eeva, Liisi and Sofia, thank you for everything. You all are superb! Finally, I want to mention the most important things in my world, Mika and our adorable girls, Isla and Seela. Girls - it is wonderful that you exist. Mika, thank you for all the love and understanding and for standing by my side.

Anne Kivimäki

Nurmijärvi, November 2019

9 REFERENCES

Actis-Goretta, L., Ottaviani, J. I., & Fraga, C. G. (2006). Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *Journal of Agricultural and Food Chemistry, 54*, 229-234.

Actis-Goretta, L., Ottaviani, J. I., Keen, C. L., & Fraga, C. G. (2003). Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. *FEBS Letters, 555*, 597-600.

Ahmetaj-Shala, B., Kirkby, N. S., Knowles, R., Al'Yamani, M., Mazi, S., Wang, Z.*, et al.* (2015). Evidence that links loss of cyclooxygenase-2 with increased asymmetric dimethylarginine: novel explanation of cardiovascular side effects associated with anti-inflammatory drugs. *Circulation, 131*, 633-642.

Al Hamimi, S., Heyman-Lindén, L., Plaza, M., Turner, C., Berger, K., & Spégel, P. (2017). Alterations in the plasma metabolite profile associated with improved hepatic function and glycemia in mice fed lingonberry supplemented high-fat diets. *Molecular Nutrition and Food Research, 61*.

Amani, R., Moazen, S., Shahbazian, H., Ahmadi, K., & Jalali, M. T. (2014). Flavonoid-rich beverage effects on lipid profile and blood pressure in diabetic patients. *World Journal of Diabetes, 5*, 962-968.

An, S.J., Boyd, R., Zhu, M., Chapman, A., Pimentel, D. R., & Wang, H. D. (2007). NADPH oxidase mediates angiotensin II-induced endothelin-1 expression in vascular adventitial fibroblasts. *Cardiovascular Research, 75*, 702-709.

Appel, L.J., Moore, T. J., Obarzanek, E., Vollmer, W. M., Svetkey, L. P., Sacks, F. M.*, et al.* (1997). A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *The New England Journal of Medicine, 336*, 1117-1124.

Arevström, L., Bergh, C., Landberg, R., Wu, H., Rodriguez-Mateos, A., Waldenborg, M.*, et al.* (2019). Freeze-dried bilberry (*Vaccinium myrtillus*) dietary supplement improves walking distance and lipids after myocardial infarction: an open-label randomized clinical trial. *Nutrition Research, 62*, 13-22.

Auch-Schwelk, W., Bossaller, C., Claus, M., Graf, K., Grafe, M., & Fleck, E. (1993). ACE inhibitors are endothelium dependent vasodilators of coronary arteries during submaximal stimulation with bradykinin. *Cardiovascular Research, 27*, 312-317.

Auger, C., Kim, J., Trinh, S., Chataigneau, T., Popken, A. M., & Schini-Kerth, V. B. (2011). Fruit juice-induced endothelium-dependent relaxations in isolated porcine coronary arteries: Evaluation of different fruit juices and purees and optimization of a red fruit juice blend. *Food and Function, 2*, 245-250.

Auger, C., Pollet, B., Arnold, C., Marx, C., & Schini-Kerth, V. B. (2015). Great heterogeneity of commercial fruit juices to induce endothelium-dependent relaxations in isolated porcine

coronary Arteries: Role of the phenolic content and composition. *Journal of Medicinal Food, 18*, 128-136.

Bachmann, S., Bosse, H. M., & Mundel, P. (1995). Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. *American Journal of Physiology-Renal Physiology, 268*, F885-F898.

Basheer, L., & Kerem, Z. (2015). Interactions between CYP3A4 and dietary polyphenols. *Oxidative Medicine and Cellular Longevity, 2015*, 854015.

Basu, A., Betts, N. M., Nguyen, A., Newman, E. D., Fu, D., & Lyons, T. J. (2014). Freeze-dried strawberries lower serum cholesterol and lipid peroxidation in adults with abdominal adiposity and elevated serum lipids. *The Journal of Nutrition, 144*, 830-837.

Basu, A., Du, M., Leyva, M. J., Sanchez, K., Betts, N. M., Wu, M*., et al.* (2010). Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *Journal of Nutrition, 140*, 1582-1587.

Basu, A., Fu, D. X., Wilkinson, M., Simmons, B., Wu, M., Betts, N. M*., et al.* (2010). Strawberries decrease atherosclerotic markers in subjects with metabolic syndrome. *Nutrition research, 30*, 462-469.

Bedada, S. K., Yellu, N. R., and Neerati, P. (2016) Effect of resveratrol treatment on the pharmacokinetics of diclofenac in healthy human volunteers. *Phytotherapy Research*, 30, 397– 401

Benjamin, E.J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S*., et al.* (2018). Heart disease and stroke statistics-2018 Update: A report from the American Heart Association. *Circulation, 137*, e67-e492.

Berk, B.C., Min, W., Yan, C., Surapisitchat, J., Liu, Y., & Hoefen, R. (2002). Atheroprotective mechanisms activated by fluid shear stress in endothelial cells. *Drug news & perspectives, 15*, 133-139.

Blake, G.J., Rifai, N., Buring, J. E., & Ridker, P. M. (2003). Blood pressure, C-reactive protein, and risk of future cardiovascular events. *Circulation, 108*, 2993-2999.

Borghi, C., & Cicero, A. F. (2017). Nutraceuticals with a clinically detectable blood pressure-lowering effect: a review of available randomized clinical trials and their meta-analyses. *British Journal of Clinical Pharmacology, 83*, 163-171.

Brader, L., Uusitupa, M., Dragsted, L. O., & Hermansen, K. (2014). Effects of an isocaloric healthy Nordic diet on ambulatory blood pressure in metabolic syndrome: A randomized SYSDIET sub-study. *European Journal of Clinical Nutrition, 68*, 57-63.

Breyer, M.D., Hao, C., & Qi, Z. (2001). Cyclooxygenase-2 selective inhibitors and the kidney. *Current Opinion in Critical Care, 7*, 393-400.

Breyer, M.D., & Harris, R. C. (2001). Cyclooxygenase 2 and the kidney. *Current Opinion in Nephrology and Hypertension, 10*, 89-98.

Briet, M., & Schiffrin, E. L. (2013). Vascular actions of aldosterone. *Journal of Vascular Research, 50*, 89-99.

Bruneval, P., Hinglais, N., Alhenc-Gelas, F., Tricottet, V., Corvol, P., Menard, J.*, et al.* (1986). Angiotensin I converting enzyme in human intestine and kidney. Ultrastructural immunohistochemical localization. *Histochemistry, 85*, 73-80.

Bujor, O., Ginies, C., Popa, V. I., & Dufour, C. (2018). Phenolic compounds and antioxidant activity of lingonberry (*Vaccinium vitis-idaea L*.) leaf, stem and fruit at different harvest periods. *Food Chemistry, 252*, 356-365.

Cahill, P.A., & Redmond, E. M. (2016). Vascular endothelium - Gatekeeper of vessel health. *Atherosclerosis, 248*, 97-109.

Cappuccio, F.P., Beer, M., Strazzullo, P., & European Salt Action Network (2018). Population dietary salt reduction and the risk of cardiovascular disease. A scientific statement from the European Salt Action Network. *Nutrition, metabolism, and cardiovascular diseases : NMCD, 29*, 107-114.

Casarini, D.E., Boim, M. A., Stella, R. C., .Krieger-Azzolini, M., Krieger,J.E, & Schor, N. (1997). Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. *The American Journal of Physiology, 272*, F405-409.

Cassidy, A, Bertoia, M., Chiuve, S., Flint, A., Forman, J., & Rimm, E.B. (2016). Habitual intake of anthocyanins and flavanones and risk of cardiovascular disease in men. American Journal of Clinical Nutrition, 104, 587-594.

Castañeda-Arriaga, R., Pérez-González, A., Reina, M., Alvarez-Idaboy, J.R. & Galano, A. (2018) Comprehensive investigation of the antioxidant and pro-oxidant effects of phenolic compounds: A double-edged sword in the context of oxidative stress? *The Journal of Physical Chemistry*, 122, 6198-6214.

Cheng, H.F., Wang, J. L., Zhang, M. Z., McKanna, J. A., & Harris, R. C. (2000). Nitric oxide regulates renal cortical cyclooxygenase-2 expression. *American Journal of Physiology - Renal physiology, 279*, F122-9.

Cheng, H.F., Wang, J. L., Zhang, M. Z., Miyazaki, Y., Ichikawa, I., McKanna, J. A.*, et al.* (1999). Angiotensin II attenuates renal cortical cyclooxygenase-2 expression. *The Journal of Clinical Investigation, 103*, 953-961.

Cicero, A.F., Gerocarni, B., Laghi, L., & Borghi, C. (2011). Blood pressure lowering effect of lactotripeptides assumed as functional foods: a meta-analysis of current available clinical trials. *Journal of Human Hypertension, 25*, 425-436.

Cook, N.R., Appel, L. J., & Whelton, P. K. (2016). Sodium Intake and all-cause mortality over 20 years in the trials of hypertension prevention. *Journal of the American College of Cardiology, 68*, 1609-1617.

Cortez, A.F., Muxfeldt, E. S., Cardoso, C. R. L., & Salles, G. F. (2016). Prognostic value of C-reactive protein in resistant hypertension. *American Journal of Hypertension, 29*, 992-1000.

Culver, S., Li, C., & Siragy, H. M. (2017). Intrarenal angiotensin-converting enzyme: the old and the new. *Current Hypertension Reports, 19*, 80.

Current Care GuidelinesHypertension. Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim and the Finnish Cardiac Society.The Finnish Medical Society Duodecim, 2014 (referred April 30, 2019). Available online at: www.kaypahoito.fi Hypertension.

Curtis, E., Fuggle, N., Shaw, S., Spooner, L., Ntani, G., Parsons, C*., et al.* (2019). Safety of cyclooxygenase-2 inhibitors in osteoarthritis: outcomes of a systematic review and meta-Analysis. *Drugs & Aging, 36*, 25-44.

Curtis, P.J., van der Velpen, V., Berends, L., Jennings, A., Feelisch, M., Umpleby, A. M*., et al.* (2019). Blueberries improve biomarkers of cardiometabolic function in participants with metabolic syndrome-results from a 6-month, double-blind, randomized controlled trial. *The American Journal of Clinical Nutrition, 109*, 1535-1545.

Czank, C., Cassidy, A., Zhang, Q., Morrison, D. J., Preston, T., Kroon, P. A*., et al.* (2013). Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. *The American Journal of Clinical Nutrition, 97*, 995-1003.

Dalgård, C., Nielsen, F., Morrow, J. D., Enghusen-Poulsen, H., Jonung, T., Hørder, M*., et al.* (2009). Supplementation with orange and blackcurrant juice, but not vitamin E, improves inflammatory markers in patients with peripheral arterial disease. *British Journal of Nutrition, 101*, 263-269.

Damera, S., Raphael, K. L., Baird, B. C., Cheung, A. K., Greene, T., & Beddhu, S. (2011). Serum alkaline phosphatase levels associate with elevated serum C-reactive protein in chronic kidney disease. *Kidney International, 79*, 228-233.

Davis, J.O., & Freeman, R. H. (1976). Mechanisms regulating renin release. *Physiological Reviews, 56*, 1-56.

de Ferrars, R.M., Czank, C., Zhang, Q., Botting, N. P., Kroon, P. A., Cassidy, A*., et al.* (2014). The pharmacokinetics of anthocyanins and their metabolites in humans. *British Journal of Pharmacology, 171*, 3268-3282.

Del Rio, D., Borges, G., & Crozier, A. (2010). Berry flavonoids and phenolics: bioavailability and evidence of protective effects. *The British Journal of Nutrition, 104 Suppl 3*, S67-90.

Dogan, M.F., Yildiz, O., Arslan, S. O., & Ulusoy, K. G. (2019). Potassium channels in vascular smooth muscle: a pathophysiological and pharmacological perspective. *Fundamental & Clinical pharmacology*.

Dohadwala, M.M., Holbrook, M., Hamburg, N. M., Shenouda, S. M., Chung, W. B., Titas, M*., et al.* (2011). Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. *American Journal of Clinical Nutrition, 93*, 934-940.

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N*., et al.* (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation Research, 87*, E1-9.

Drozdz, P., Seziene, V., Wojcik, J., & Pyrzynska, K. (2017). Evaluation of bioactive bompounds, Minerals and Antioxidant Activity of Lingonberry (*Vaccinium vitis-idaea L.*) Fruits. *Molecules, 23*, 10.3390/molecules23010053.

Duka, A., Duka, I., Gao, G., Shenouda, S., Gavras, I., & Gavras, H. (2006). Role of bradykinin B1 and B2 receptors in normal blood pressure regulation. *American Journal of Physiology.Endocrinology and Metabolism, 291*, E268-74.

Edwards, R.M., & Stack, E. J. (1993). Angiotensin II inhibits glomerular adenylate cyclase via the angiotensin II receptor subtype 1 (AT1). *The Journal of Pharmacology and Experimental therapeutics, 266*, 506-510.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. (2016). Scientific opinion on the safety of synthetic trans-resveratrol as a novel food pursuant to Regulation (EC) No 258/97. EFSA Journal 2016;14(1):4368.

EFSA Panel on Dietetic Products, Nutrition,and Allergies (2012). Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006. *EFSA Journal, 10*, 2809.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (2013). Scientific Opinion on the re-evaluation of anthocyanins (E 163) as a food additive. EFSA Journal 2013;11(4):3145.

Ek, S., Kartimo, H., Mattila, S., & Tolonen, A. (2006). Characterization of phenolic compounds from lingonberry (Vaccinium vitis-idaea). *Journal of Agricultural and Food Chemistry, 54*, 9834-9842.

Erlund, I., Koli, R., Alfthan, G., Marniemi, J., Puukka, P., Mustonen, P*., et al.* (2008). Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *The American Journal of Clinical Nutrition, 87*, 323-331.

Ewart, M.A., Kohlhaas, C. F., & Salt, I. P. (2008). Inhibition of tumor necrosis factor alpha-stimulated monocyte adhesion to human aortic endothelial cells by AMP-activated protein kinase. *Arteriosclerosis, Thrombosis, and Vascular Biology, 28*, 2255-2257.

Fabricio, V., Oishi, J. C., Biffe, B. G., Ruffoni, L. D., Silva, K. A., Nonaka, K. O*., et al.* (2017). Resveratrol treatment normalizes the endothelial function and blood pressure in ovariectomized rats. *Arquivos Brasileiros de Cardiologia, 108*, 116-121.

Feresin, R.G., Johnson, S. A., Pourafshar, S., Campbell, J. C., Jaime, S. J., Navaei, N*., et al.* (2017). Impact of daily strawberry consumption on blood pressure and arterial stiffness in pre- and stage 1-hypertensive postmenopausal women: a randomized controlled trial. *Food & Function, 8*, 4139-4149.

Filippini, T., Violi, F., D'Amico, R., & Vinceti, M. (2017). The effect of potassium supplementation on blood pressure in hypertensive subjects: A systematic review and meta-analysis. *International Journal of Cardiology, 230*, 127-135.

Fishman, A.P. (1982). Endothelium: a distributed organ of diverse capabilities. *Annals of the New York Academy of Sciences, 401*, 1-8.

Foxman, B., Cronenwett, A. E., Spino, C., Berger, M. B., & Morgan, D. M. (2015). Cranberry juice capsules and urinary tract infection after surgery: results of a randomized trial. *American Journal of Obstetrics and Gynecology, 213*, 194.e1-194.e8.

Furchgott, R.F., & Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature 288, 373-6*.

Furusu, A., Miyazaki, M., Abe, K., Tsukasaki, S., Shioshita, K., Sasaki, O*., et al.* (1998). Expression of endothelial and inducible nitric oxide synthase in human glomerulonephritis. *Kidney International, 53*, 1760-1768.

Fyhrquist, F., & Saijonmaa, O. (2008). Renin-angiotensin system revisited. *Journal of Internal medicine, 264, 224-36*.

Garcia-Cohen, E.C., Marin, J., Diez-Picazo, L. D., Baena, A. B., Salaices, M., & Rodriguez-Martinez, M. A. (2000). Oxidative stress induced by tert-butyl hydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: role of cyclooxygenase-2 isoform. *The Journal of Pharmacology and Experimental Therapeutics, 293*, 75-81.

Goszcz, K., Duthie, G. G., Stewart, D., Leslie, S. J., & Megson, I. L. (2017). Bioactive polyphenols and cardiovascular disease: chemical antagonists, pharmacological agents or xenobiotics that drive an adaptive response? *British Journal of Pharmacology, 174*, 1209-1225.

Graves, J., & Poston, L. (1993). Beta-adrenoceptor agonist mediated relaxation of rat isolated resistance arteries: a role for the endothelium and nitric oxide. *British Journal of Pharmacology, 108*, 631-637.

Guo, X., Yang, B., Cai, W., & Li, D. (2017). Effect of sea buckthorn (Hippophae rhamnoides L.) on blood lipid profiles: A systematic review and meta-analysis from 11 independent randomized controlled trials. *Trends in Food Science & Technology, 61*, 1-10.

Guyenet, P.G. (2006). The sympathetic control of blood pressure. *Nature Reviews.Neuroscience, 7*, 335-346.

Guyton, A.C., Coleman, T. G., Cowley, A. V.,Jr, Scheel, K. W., Manning, R. D.,Jr, & Norman, R. A.,Jr (1972). Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *The American Journal of Medicine, 52*, 584-594.

Guzik, T.J., & Touyz, R. M. (2017). Oxidative Stress, inflammation, and vascular aging in hypertension. *Hypertension, 70*, 660-667.

Haarhaus, M., Brandenburg, V., Kalantar-Zadeh, K., Stenvinkel, P., & Magnusson, P. (2017). Alkaline phosphatase: a novel treatment target for cardiovascular disease in CKD. *Nature Reviews.Nephrology, 13*, 429-442.

Häkkinen, S.H., Kärenlampi, S.O., Heinonen, M.I., Mykkänen, H.M. & Törrönen, A.R. (1999) Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries *Journal of Agricultural and Food Chemistry.* 47, 2274-2279.

Hall, J.E. & Guyton, A.C. (2011a) Chapter 18: Nervous regulation of the circulation, and rapid control of arterial pressure. In: Guyton and Hall Textbook of Medical Physiology. Philadelphia, Pa Saunders, 201-211.

Hall, J.E. & Guyton, A.C. (2011b) Chapter 60: The autonomic nervous system and the adrenal medulla. In: Guyton and Hall Textbook of Medical Physiology. Philadelphia, Pa Saunders, 729-741.

Hämäläinen, M., Nieminen, R., Vuorela, P., Heinonen, M., & Moilanen, E. (2007). Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators of Inflammation, 2007*, 45673.

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *The Journal of Pathology, 203*, 631-637.

Harbaoui, B., Courand, P. Y., Defforges, A., Khettab, F., Milon, H., Girerd, N*., et al.* (2016). Cumulative effects of several target organ damages in risk assessment in hypertension. *American Journal of Hypertension, 29*, 234-244.

Harris, R.C. (2006). COX-2 and the kidney. *Journal of Cardiovascular Pharmacology, 47 Suppl 1*, S37-42.

Harris, R.C., & Breyer, M. D. (2001). Physiological regulation of cyclooxygenase-2 in the kidney. *American journal of physiology.Renal Physiology, 281*, F1-11.

Harris, R.C., McKanna, J. A., Akai, Y., Jacobson, H. R., Dubois, R. N., & Breyer, M. D. (1994). Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *Journal of Clinical Investigation, 94*, 2504-2510.

Harris, R.C., Zhang, M. Z., & Cheng, H. F. (2004). Cyclooxygenase-2 and the renal renin-angiotensin system. *Acta Physiologica Scandinavica, 181*, 543-547.

He, F.J., Li, J., & Macgregor, G. A. (2013). Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *British Medical Journal (Clinical research ed.), 346*, f1325.

Hellström, J.K., Törrönen, R.A., Mattila, P.H. (2009). Proanthocyanins in common food products of plant origin. *Journal of Agricultural and Food Chem*istry, 57, 7899-7906

Hemler, M.E., & Lands, W. E. (1977). Biosynthesis of prostaglandins. *Lipids, 12*, 591-595.

Heneghan, C., Kiely, M., Lyons, J., & Lucey, A. (2018). The effect of berry-based food interventions on markers of cardiovascular and metabolic health: A systematic review of randomized controlled trials. *Molecular Nutrition & Food Research, 62*, 1700645-n/a.

Heyman, L., Axling, U., Blanco, N., Sterner, O., Holm, C., & Berger, K. (2014). Evaluation of beneficial metabolic effects of berries in high-fat fed C57BL/6J mice. *Journal of Nutrition and Metabolism, 2014*.

Heyman-Linden, L., Seki, Y., Storm, P., Jones, H. A., Charron, M. J., Berger, K*., et al.* (2016). Berry intake changes hepatic gene expression and DNA methylation patterns associated with high-fat diet. *The Journal of Nutritional Biochemistry, 27*, 79-95.

Hjartåker, A., Knudsen, M. D., Tretli, S., & Weiderpass, E. (2015). Consumption of berries, fruits and vegetables and mortality among 10,000 Norwegian men followed for four decades. *European Journal of Nutrition, 54*, 599-608.

Holappa, M., Vapaatalo, H., & Vaajanen, A. (2017). Many faces of renin-angiotensin system - focus on eye. *The Open Ophthalmology Journal, 11*, 122-142.

Hooper, N.M., & Turner, A. J. (2003). An ACE structure. *Nat Struct Mol Biol, 10*, 155-157.

Houston, M.C., & Harper, K. J. (2008). Potassium, magnesium, and calcium: their role in both the cause and treatment of hypertension. *Journal of Clinical Hypertension, 10*, 3-11.

Hsieh, H.J., Liu, C. A., Huang, B., Tseng, A. H., & Wang, D. L. (2014). Shear-induced endothelial mechanotransduction: the interplay between reactive oxygen species (ROS) and nitric oxide (NO) and the pathophysiological implications. *Journal of Biomedical Science, 21*, 3-0127-21-3.

Hykkerud,A.L., Uleberg,E., Hansen,E., Vervoort,M., Mølmann,J. & Martinussen,I. (2018) Seasonal and yearly variation of total polyphenols, total anthocyanins and ellagic acid in different clones of cloudberry (Rubus chamaemorus L.) *Journal of Applied Botany and Food Quality*, 91, 96-102.

Ignarro, L.J., Buga, G. M., Wood, K. S., Byrns, R. E., & Chaudhuri, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America, 84*, 9265-9269.

Ignarro, L.J., Harbison, R. G., Wood, K. S., & Kadowitz, P. J. (1986). Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: stimulation by acetylcholine, bradykinin and arachidonic acid. *The Journal of Pharmacology and Experimental Therapeutics, 237*, 893-900.

Incalza, M.A., D'Oria, R., Natalicchio, A., Perrini, S., Laviola, L., & Giorgino, F. (2018). Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascular Pharmacology, 100*, 1-19.

Isaak, C.K., Petkau, J. C., Blewett, H., Karmin, O., & Siow, Y. L. (2017). Lingonberry anthocyanins protect cardiac cells from oxidative-stress-induced apoptosis. *Canadian Journal of Physiology and Pharmacology, 95*, 904-910.

Ivy, J.R., & Bailey, M. A. (2014). Pressure natriuresis and the renal control of arterial blood pressure. *The Journal of Physiology, 592*, 3955-3967.

Jang, I.A., Kim, E. N., Lim, J. H., Kim, M. Y., Ban, T. H., Yoon, H. E.*, et al.* (2018). Effects of Resveratrol on the Renin-Angiotensin System in the Aging Kidney. *Nutrients, 10*, 10.3390.

Jayedi, A., & Zargar, M. S. (2018). Dietary calcium intake and hypertension risk: a dose-response meta-analysis of prospective cohort studies. *European Journal of Clinical nutrition, 10*.

Jennings, A., Welch, A. A., Fairweather-Tait, S. J., Kay, C., Minihane, A., Chowienczyk, P.*, et al.* (2012). Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. *American Journal of Clinical Nutrition, 96*, 781-788.

Johansson, A.K., Korte, H., Yang, B., Stanley, J. C., & Kallio, H. P. (2000). Sea buckthorn berry oil inhibits platelet aggregation. *The Journal of Nutritional Biochemistry, 11*, 491-495.

Johnson, S.A., Figueroa, A., Navaei, N., Wong, A., Kalfon, R., Ormsbee, L. T.*, et al.* (2015). Daily blueberry consumption improves blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension: A randomized, double-blind, placebo-controlled clinical trial. *Journal of the Academy of Nutrition and Dietetics, 115*, 369-377.

Jourde-Chiche, N., Fakhouri, F., Dou, L., Bellien, J., Burtey, S., Frimat, M.*, et al.* (2019). Endothelium structure and function in kidney health and disease. *Nature Reviews.Nephrology, 15*, 87-108.

Juraschek, S.P., Miller, E. R.,3rd, Weaver, C. M., & Appel, L. J. (2017). Effects of sodium reduction and the DASH diet in relation to baseline blood pressure. *Journal of the American College of Cardiology, 70*, 2841-2848.

Kardum, N., & Glibetic, M. (2018). Polyphenols and their interactions with other dietary compounds: implications for human health. *Advances in Food and Nutrition Research, 84*, 103-144.

Kardum, N., Milovanovic, B., Šavikin, K., Zdunic, G., Mutavdžin, S., Gligorijevic, T.*, et al.* (2015). Beneficial effects of polyphenol-rich chokeberry juice consumption on blood pressure level and lipid status in hypertensive subjects. *Journal of Medicinal Food, 18*, 1231-1238.

Karlsen, A., Paur, I., Bøhn, S. K., Sakhi, A. K., Borge, G. I., Serafini, M.*, et al.* (2010). Bilberry juice modulates plasma concentration of NF-κB related inflammatory markers in subjects at increased risk of CVD. *European Journal of Nutrition, 49*, 345-355.

Kass, L., Weekes, J., & Carpenter, L. (2012). Effect of magnesium supplementation on blood pressure: a meta-analysis. *European Journal of Clinical Nutrition, 66*, 411-418.

Kay, C.D., Hooper, L., Kroon, P. A., Rimm, E. B., & Cassidy, A. (2012). Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products. *Molecular Nutrition and Food Research, 56*, 1605-1616.

Khan, F., Ray, S., Craigie, A. M., Kennedy, G., Hill, A., Barton, K. L*., et al.* (2014). Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: A randomized controlled trial of antioxidant- and polyphenol-rich blackcurrant juice. *Free Radical Biology and Medicine, 72*, 232-237.

Khanna, A., English, S. W., Wang, X. S., Ham, K., Tumlin, J., Szerlip, H*., et al.* (2017). Angiotensin II for the treatment of vasodilatory Shock. *The New England Journal of Medicine, 377*, 419-430.

Kimble, R., Keane, K. M., Lodge, J. K., & Howatson, G. (2018). Dietary intake of anthocyanins and risk of cardiovascular disease: A systematic review and meta-analysis of prospective cohort studies. *Critical Reviews in Food Science and Nutrition*, 1-12.

Kirkby, N.S., Reed, D. M., Edin, M. L., Rauzi, F., Mataragka, S., Vojnovic, I*., et al.* (2015). Inherited human group IVA cytosolic phospholipase A2 deficiency abolishes platelet, endothelial, and leucocyte eicosanoid generation. *FASEB journal, 29*, 4568-4578.

Kirkby, N.S., Sampaio, W., Etelvino, G., Alves, D. T., Anders, K. L., Temponi, R*., et al.* (2018). Cyclooxygenase-2 selectively controls renal blood flow through a novel PPARbeta/delta-dependent vasodilator pathway. *Hypertension, 71*, 297-305.

Kirkby, N.S., Zaiss, A. K., Urquhart, P., Jiao, J., Austin, P. J., Al-Yamani, M*., et al.* (2013). LC-MS/MS confirms that COX-1 drives vascular prostacyclin whilst gene expression pattern reveals non-vascular sites of COX-2 expression. *PloS one, 8*, e69524.

Kobori, H., Nangaku, M., Navar, L. G., & Nishiyama, A. (2007). The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacological Reviews, 59*, 251-287.

Kolehmainen, M., Mykkänen, O., Kirjavainen, P. V., Leppänen, T., Moilanen, E., Adriaens, M*., et al.* (2012). Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. *Molecular Nutrition and Food Research, 56*, 1501-1510.

Koponen, J.M., Happonen, A.M., Mattila, P.H. & Törrönen, A.R. (2007) Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *Journal of Agricultural and Food Chemistry,* 55, 1612-1619.

Kuba, K., Imai, Y., Ohto-Nakanishi, T., & Penninger, J. M. (2010). Trilogy of ACE2: A peptidase in the renin–angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacology & Therapeutics, 128*, 119-128.

Kujubu, D.A., Fletcher, B. S., Varnum, B. C., Lim, R. W., & Herschman, H. R. (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *The Journal of Biological Chemistry, 266*, 12866-12872.

Kumpusalo, E., Lappi, J., Miettinen, H., & Takala, J. (2001). Prevalence of left ventricular hypertrophy in Finnish primary health care hypertensive patients. *Journal of Human Hypertension, 15*, 255-258.

Kuoppala, A., Shiota, N., Kokkonen, J. O., Liesmaa, I., Kostner, K., Mayranpaa, M.*, et al.* (2002). Down-regulation of cardioprotective bradykinin type-2 receptors in the left ventricle of patients with end-stage heart failure. *Journal of the American College of Cardiology, 40*, 119-125.

Kurtz, A. (2011). Renin release: sites, mechanisms, and control. *Annual Review of Physiology, 73*, 377-399.

Kurtz, A., & Penner, R. (1989). Angiotensin II induces oscillations of intracellular calcium and blocks anomalous inward rectifying potassium current in mouse renal juxtaglomerular cells. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 3423-3427.

Laatikainen, T., Jula, A., & Salomaa, V. (2012). *Verenpaine Suomessa : FINRISKI-tutkimuksen tuloksia.* Terveyden ja hyvinvoinnin laitos: Helsinki.

Laitinen, T.T., Ruohonen, S., Juonala, M., Magnussen, C. G., Mikkila, V., Mikola, H.*, et al.* (2017). Ideal cardiovascular health in childhood-Longitudinal associations with cardiac structure and function: The Special Turku Coronary Risk Factor Intervention Project (STRIP) and the Cardiovascular Risk in Young Finns Study (YFS). *International Journal of Cardiology, 230*, 304-309.

Landowne, M., Thompson, W.,Jr, & Ruby, B. (1949). Minimal sodium diet; a controlled study of its effect upon the blood pressure of ambulatory hypertensive subjects. *The American Journal of Medicine, 6*, 664.

Langer S. & Hicks P. (1984). Alpha-adrenoreceptor subtypes in blood vessels: Physiology and pharmacology. *Journal od Cardiovascular Pharmacology*, 6, 547-558.

Larsson, S.C., Virtamo, J., & Wolk, A. (2013). Total and specific fruit and vegetable consumption and risk of stroke: a prospective study. *Atherosclerosis, 227*, 147-152.

Lehtonen, H.M., Rantala, M., Suomela, J. P., Viitanen, M., & Kallio, H. (2009). Urinary excretion of the main anthocyanin in lingonberry (*Vaccinium vitis-idaea*), cyanidin 3-O-galactoside, and its metabolites. *Journal of Agricultural and Food Chemistry, 57*, 4447-4451.

Li, X., Dai, Y., Yan, S., Shi, Y., Li, J., Liu, J.*, et al.* (2016). Resveratrol lowers blood pressure in spontaneously hypertensive rats via calcium-dependent endothelial NO production. *Clinical and Experimental Hypertension, 38*, 287-293.

Liesmaa, I., Kuoppala, A., Shiota, N., Kokkonen, J. O., Kostner, K., Mäyränpää, M.*, et al.* (2005). Increased expression of bradykinin type-1 receptors in endothelium of intramyocardial coronary vessels in human failing hearts. *American Journal of Physiology.Heart and Circulatory Physiology, 288*, H2317-22.

Linderborg, K.M., Jarvinen, R., Lehtonen, H. M., Viitanen, M., & Kallio, H. P. (2012). The fiber and/or polyphenols present in lingonberries null the glycemic effect of the sugars present in the berries when consumed together with added glucose in healthy human volunteers. *Nutrition Research, 32*, 471-478.

Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-kappaB signaling in inflammation. *Signal Transduction and Targeted Therapy, 2*, 10,1038.

Livak, K.J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods, 25*, 402-408.

Lohmeier, T.E., & Iliescu, R. (2015). The baroreflex as a long-term controller of arterial pressure. *Physiology, 30*, 148-158.

Loo, B., Erlund, I., Koli, R., Puukka, P., Hellström, J., Wähälä, K.*, et al.* (2016). Consumption of chokeberry (*Aronia mitschurinii*) products modestly lowered blood pressure and reduced low-grade inflammation in patients with mildly elevated blood pressure. *Nutrition Research, 36*, 1222-1230.

Määttä-Riihinen, K.R., Kähkönen, M. P., Törrönen, A. R., & Heinonen, I. M. (2005). Catechins and procyanidins in berries of Vaccinium species and their antioxidant activity. *Journal of Agricultural and Food Chemistry, 53*, 8485-8491.

Majesky, M.W. (2016). Vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology, 36*, e82-6.

Mäkynen, H., Arvola, P., Vapaatalo, H., & Pörsti, I. (1994). High calcium diet effectively opposes the development of deoxycorticosterone-salt hypertension in rats. *American Journal of Hypertension, 7*, 520-528.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition, 79*, 727-747.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition, 81*, 230S-242S.

Mantelli, L., Amerini, S., & Ledda, F. (1995). Bradykinin-induced vasodilation is changed to a vasoconstrictor response in vessels of aged normotensive and hypertensive rats. *Inflammation research, 44*, 70-73.

Mantzorou, M., & Giaginis, C. (2018). Cranberry consumption against urinary tract infections: clinical stateof- the-art and future perspectives. *Current Pharmaceutical Biotechnology, 19*, 1049-1063.

Marcondes, S., Cardoso, M. H., Morganti, R. P., Thomazzi, S. M., Lilla, S., Murad, F.*, et al.* (2006). Cyclic GMP-independent mechanisms contribute to the inhibition of platelet adhesion by nitric oxide donor: a role for alpha-actinin nitration. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 3434-3439.

Marin, L., Miguelez, E. M., Villar, C. J., & Lombo, F. (2015). Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. *BioMedical Research International,* 905215.

Matziouridou, C., Marungruang, N., Nguyen, T. D., Nyman, M., & Fåk, F. (2016). Lingonberries reduce atherosclerosis in Apoe-/- mice in association with altered gut microbiota composition and improved lipid profile. *Molecular Nutrition and Food Research, 60*, 1150-1160.

McAnulty, L.S., Collier, S. R., Landram, M. J., Whittaker, D. S., Isaacs, S. E., Klemka, J. M.*, et al.* (2014). Six weeks daily ingestion of whole blueberry powder increases natural killer cell counts and reduces arterial stiffness in sedentary males and females. *Nutrition Research, 34*, 577-584.

Medina-Remon, A., Tresserra-Rimbau, A., Pons, A., Tur, J. A., Martorell, M., Ros, E.*, et al.* (2015). Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. *Nutrition, Metabolism, and Cardiovascular Diseases : NMCD, 25*, 60-67.

Mendonca, R.D., Carvalho, N. C., Martin-Moreno, J. M., Pimenta, A. M., Lopes, A. C. S., Gea, A.*, et al.* (2019). Total polyphenol intake, polyphenol subtypes and incidence of cardiovascular disease: The SUN cohort study. *Nutrition, Metabolism, and Cardiovascular Diseases : NMCD, 29*, 69-78.

Miller, A.J., & Arnold, A. C. (2018). The renin-angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. *Clinical Autonomic Research*, 2, 231-243

Mitchell, J.A., & Kirkby, N. S. (2019). Eicosanoids, prostacyclin and cyclooxygenase in the cardiovascular system. *British Journal of Pharmacology, 176*, 1038-1050.

Mitjavila, M.T., Fandos, M., Salas-Salvado, J., Covas, M. I., Borrego, S., Estruch, R.*, et al.* (2013). The Mediterranean diet improves the systemic lipid and DNA oxidative damage in metabolic syndrome individuals. A randomized, controlled, trial. *Clinical Nutrition, 32*, 172-178.

Moncada, S., & Higgs, E. A. (2006). The discovery of nitric oxide and its role in vascular biology. *British Journal of Pharmacology, 147 Suppl 1*, S193-201.

Moncada, S., & Vane, J. R. (1978). Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2, and prostacyclin. *Pharmacological Reviews, 30*, 293-331.

Monica, F.Z., Bian, K., & Murad, F. (2016). The endothelium-dependent nitric oxide-cGMP pathway. *Advances in Pharmacology, 77*, 1-27.

Montezano, A.C., Nguyen, D. C., Rios, F. J., & Touyz, R. M. (2014). Angiotensin II and vascular injury. *Current Hypertension Reports, 16*, 431.

Mozos, I., Malainer, C., Horbanczuk, J., Gug, C., Stoian, D., Luca, C. T.*, et al.* (2017). Inflammatory markers for arterial stiffness in cardiovascular diseases. *Frontiers in Immunology, 8*, 1058.

Mulvany, M.J., & Aalkjaer, C. (1990). Structure and function of small arteries. *Physiological Reviews, 70*, 921-961.

Murad, F. (1986). Cyclic guanosine monophosphate as a mediator of vasodilation. *The Journal of Clinical Investigation, 78*, 1-5.

Mursu, J., Virtanen, J. K., Tuomainen, T. P., Nurmi, T., & Voutilainen, S. (2014). Intake of fruit, berries, and vegetables and risk of type 2 diabetes in Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *The American Journal of Clinical Nutrition, 99*, 328-333.

National Institute for Health and Welfare, Nutrition Unit. Fineli.Finnish food composition database. Helsinki 2018.

Nayeem, F., Anderson, K. E., Nagamani, M., Grady, J. J., & Lu, L. J. (2010). Alkaline phosphatase and percentage body fat predict circulating C-reactive protein in premenopausal women. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals, 15*, 663-670.

Nguyen D. C., A, Montezano, A. C., Burger, D., & Touyz, R. M. (2013). Angiotensin II, NADPH oxidase, and redox signaling in the vasculature. *Antioxidants & Redox Signaling, 19*, 1110-1120.

Nguyen, G., Delarue, F., Burckle, C., Bouzhir, L., Giller, T., & Sraer, J. D. (2002). Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *The Journal of Clinical Investigation, 109*, 1417-1427.

Nilsson, A., Salo, I., Plaza, M., & Björck, I. (2017). Effects of a mixed berry beverage on cognitive functions and cardiometabolic risk markers; A randomized cross-over study in healthy older adults. *PLoS ONE, 12*.

Novotny, J.A., Baer, D. J., Khoo, C., Gebauer, S. K., & Charron, C. S. (2015). Cranberry juice consumption lowers markers of cardiometabolic risk, including blood pressure and circulating c-reactive protein, triglyceride, and glucose concentrations in adults. *Journal of Nutrition, 145*, 1185-1193.

Nurmi, T., Mursu, J., Penalvo, J.L., Poulsen, H.E. & Voutilainen, S. (2010) Dietary intake and urinary excretion of lignans in Finnish men. *British Journal of Nutrition*. 103, 677-685.

Ogunrinade, O., Kameya, G. T., & Truskey, G. A. (2002). Effect of fluid shear stress on the permeability of the arterial endothelium. *Annals of Biomedical Engineering, 30*, 430-446.

Ohlmann, P., Martinez, M. C., Schneider, F., Stoclet, J. C., & Andriantsitohaina, R. (1997). Characterization of endothelium-derived relaxing factors released by bradykinin in human resistance arteries. *British Journal of Pharmacology, 121*, 657-664.

Okamoto, K., & Aoki, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japanese Circulation Journal, 27*, 282-293.

Ong, Y., Wong, T. Y., Klein, R., Klein, B. E. K., Mitchell, P., Sharrett, A. R.*, et al.* (2013). Hypertensive retinopathy and risk of stroke. *Hypertension, 62*, 706-711.

Orlando, B.J., & Malkowski, M. G. (2016). Crystal structure of rofecoxib bound to human cyclooxygenase-2. *Acta Crystallographica, 72*, 772-776.

O'Rourke, M. (1990). Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension, 15, 339-47.*

Palmer, R.M., Ferrige, A. G., & Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature, 327*, 524-526.

Park, J.B., Kang, D. Y., Yang, H. M., Cho, H. J., Park, K. W., Lee, H. Y.*, et al.* (2013). Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. *European Heart Journal, 34*, 920-931.

Patrono, C., Pugliese, F., Ciabattoni, G., Patrignani, P., Maseri, A., Chierchia, S.*, et al.* (1982). Evidence for a direct stimulatory effect of prostacyclin on renin release in man. *The Journal of Clinical Investigation, 69*, 231-239.

Peart, W.S. (1975). Renin-angiotensin system. *The New England Journal of Medicine, 292*, 302-306.

Pelkonen, O., Maenpaa, J., Taavitsainen, P., Rautio, A., & Raunio, H. (1998). Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica, 28*, 1203-1253.

Perticone, F., Perticone, M., Maio, R., Sciacqua, A., Andreucci, M., Tripepi, G.*, et al.* (2015). Serum alkaline phosphatase negatively affects endothelium-dependent vasodilation in naive hypertensive patients. *Hypertension, 66*, 874-880.

Peters, J. (2017). The (pro)renin receptor and its interaction partners. *Pflügers Archiv - European Journal of Physiology, 469*, 1245-1256.

Pinto, Y.M., Paul, M., & Ganten, D. (1998). Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovascular Research, 39*, 77-88.

Pörsti, I., Arvola, P., Wuorela, H., & Vapaatalo, H. (1992). High calcium diet augments vascular potassium relaxation in hypertensive rats. *Hypertension, 19*, 85-92.

Priest, R.M., Hucks, D., & Ward, J. P. (1997). Noradrenaline, beta-adrenoceptor mediated vasorelaxation and nitric oxide in large and small pulmonary arteries of the rat. *British Journal of Pharmacology, 122*, 1375-1384.

Quiñones, M., Miguel, M., Muguerza, B., & Aleixandre, A. (2011). Effect of a cocoa polyphenol extract in spontaneously hypertensive rats. *Food and Function, 2*, 649-653.

Rahimi, Z. (2016). The role of renin angiotensin aldosterone system genes in diabetic nephropathy. *Canadian Journal of Diabetes, 40*, 178-183.

Ramezani-Jolfaie, N., Mohammadi, M., & Salehi-Abargouei, A. (2018). The effect of healthy Nordic diet on cardio-metabolic markers: a systematic review and meta-analysis of randomized controlled clinical trials. *European Journal of Nutrition*.

Rashid, S., Idris-Khodja, N., Auger, C., Kevers, C., Pincemail, J., Alhosin, M.*, et al.* (2018). Polyphenol-rich blackcurrant juice prevents endothelial dysfunction in the mesenteric artery of cirrhotic rats with portal hypertension: role of oxidative stress and the angiotensin system. *Journal of Medicinal Food, 21*, 390-399.

Rees, A., Dodd, G. F., & Spencer, J. P. E. (2018). The effects of flavonoids on cardiovascular health: A review of human interventiontrials and implications for cerebrovascular function. *Nutrients, 10*, 10.3390.

Reichert, K.P., Schetinger, M. R. C., Gutierres, J. M., Pelinson, L. P., Stefanello, N., Dalenogare, D. P.*, et al.* (2018). Lingonberry extract provides neuroprotection by regulating the purinergic system and reducing oxidative stress in diabetic rats. *Molecular Nutrition and Food Research, 62*.

Ricciotti, E., & Fitzgerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology, 31*, 986-1000.

Ridker, P.M., Hennekens, C. H., Buring, J. E., & Rifai, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine, 342*, 836-843.

Rienks, J., Barbaresko, J., & Nothlings, U. (2017). Association of polyphenol biomarkers with cardiovascular disease and mortality risk: A systematic review and meta-analysis of observational studies. *Nutrients, 9*, 10.3390

Rimando, A.M., Kalt, W.K., Magee, J.B., Dewey, J. & Ballington. J.R. (2004) Resveratrol, pterostilbene, and piceatannol in *Vaccinium* berries. *Journal of Agricultural and Food Chemistry*. 52, 4713-4719.

Rissanen, T.H., Voutilainen, S., Virtanen, J. K., Venho, B., Vanharanta, M., Mursu, J.*, et al.* (2003). Low intake of fruits, berries and vegetables is associated with excess mortality in men: The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. *Journal of Nutrition, 133*, 199-204.

Rius, C., Abu-Taha, M., Hermenegildo, C., Piqueras, L., Cerda-Nicolas, J. M., Issekutz, A. C.*, et al.* (2010). Trans- but not cis-resveratrol impairs angiotensin-II-mediated vascular inflammation through inhibition of NF-kappaB activation and peroxisome proliferator-activated receptor-gamma upregulation. *Journal of Immunology, 185*, 3718-3727.

Robinson, L.J., Busconi, L., & Michel, T. (1995). Agonist-modulated palmitoylation of endothelial nitric oxide synthase. *The Journal of Biological Chemistry, 270*, 995-998.

Rodicio, J.L., Campo, C., & Ruilope, L. M. (1998). Microalbuminuria in essential hypertension. *Kidney International, Supplement, 54*, S51-S54.

Rodriguez-Mateos, A., Cifuentes-Gomez, T., Gonzalez-Salvador, I., Ottaviani, J. I., Schroeter, H., Kelm, M*., et al.* (2015). Influence of age on the absorption, metabolism, and excretion of cocoa flavanols in healthy subjects. *Molecular Nutrition & Food Research, 59*, 1504-1512.

Rosen, G.D., Birkenmeier, T. M., Raz, A., & Holtzman, M. J. (1989). Identification of a cyclooxygenase-related gene and its potential role in prostaglandin formation. *Biochemical and Biophysical Research Communications, 164*, 1358-1365.

Ruel, G., Lapointe, A., Pomerleau, S., Couture, P., Lemieux, S., Lamarche, B*., et al.* (2013). Evidence that cranberry juice may improve augmentation index in overweight men. *Nutrition Research, 33*, 41-49.

Ruel, G., Pomerleau, S., Couture, P., Lemieux, S., Couillard, C., & Couillard, C. (2009). Plasma matrix metalloproteinase (mmp)-9 levels are reduced following low-calorie cranberry juice supplementation in men. *Journal of the American College of Nutrition, 28*, 694-701.

Sack, M.N., Fyhrquist, F. Y., Saijonmaa, O. J., Fuster, V., & Kovacic, J. C. (2017). Basic Biology of Oxidative Stress and the Cardiovascular System: Part 1 of a 3-Part Series. *Journal of the American College of Cardiology, 70*, 196-211.

Santos, R.A.S., Krieger, E. M., & Greene, L. J. (1985). An improved fluorometric assay of rat serum and plasma converting enzyme. *Hypertension, 7*, 244-252.

Savoia, C., & Schiffrin, E. L. (2006). Inflammation in hypertension. *Current Opinion in Nephrology and Hypertension, 15*, 152-158.

Seo, M.S., Shim, J. Y., & Lee, Y. J. (2019). Relationship between serum alkaline phosphatase level, C-reactive protein and leukocyte counts in adults aged 60 years or older. *Scandinavian Journal of Clinical and Laboratory Investigation*, 1-5.

Sheflin, A.M., Melby, C. L., Carbonero, F., & Weir, T. L. (2017). Linking dietary patterns with gut microbial composition and function. *Gut Microbes, 8*, 113-129.

Shi, Y., & Vanhoutte, P. M. (2008). Oxidative stress and COX cause hyper-responsiveness in vascular smooth muscle of the femoral artery from diabetic rats. *British Journal of Pharmacology, 154*, 639-651.

Shimokawa, H. (2010). Hydrogen peroxide as an endothelium-derived hyperpolarizing factor. *Pflugers Archiv : European Journal of Physiology, 459*, 915-922.

Siltari, A., Korpela, R., & Vapaatalo, H. (2016). Bradykinin -induced vasodilatation: Role of age, ACE1-inhibitory peptide, mas- and bradykinin receptors. *Peptides, 85*, 46-55.

Siltari, A., Roivanen, J., Korpela, R., & Vapaatalo, H. (2017). Long-term feeding with bioactive tripeptides in aged hypertensive and normotensive rats: Special focus on blood pressure and bradykinin-induced vascular reactivity. *Journal of Physiology and Pharmacology, 68*, 407-418.

Siltari A. 2018. Antihypertensive tripeptides and vasculature - Focus on mecnanisms, ageing, stereoisomer conformation and intestinal permeability. http://urn.fi/URN:ISBN:978-951-51-3962-7

Soubrier, F., Alhenc-Gelas, F., Hubert, C., Allegrini, J., John, M., Tregear, G.*, et al.* (1988). Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 9386-9390.

Stalmach, A., Williamson, G., & Crozier, A. (2014). Impact of dose on the bioavailability of coffee chlorogenic acids in humans. *Food & Function, 5*, 1727-1737.

Streatfeild-James, R.M., Williamson, D., Pike, R. N., Tewksbury, D., Carrell, R. W., & Coughlin, P. B. (1998). Angiotensinogen cleavage by renin: importance of a structurally constrained N-terminus. *FEBS letters, 436*, 267-270.

Stull, J.A., Cash, C. K., Champagne, M. C., Gupta, K. A., Boston, R., Beyl, A. R.*, et al.* (2015). Blueberries improve endothelial function, but not blood pressure, in adults with metabolic syndrome: a randomized, double-blind, placebo-controlled clinical trial. *Nutrients, 7*, 4107-4123

Szerafin, T., Erdei, N., Fulop, T., Pasztor, E. T., Edes, I., Koller, A.*, et al.* (2006). Increased cyclooxygenase-2 expression and prostaglandin-mediated dilation in coronary arterioles of patients with diabetes mellitus. *Circulation Research, 99*, e12-7.

Taal, M. W., Yu, A. S. L., Brenner, B. M., Skorecki, K., Chertow, G. M., & Marsden, P. A. (2011). *Brenner and Rector's The Kidney.* Saunders: London.

Tabart, J., Auger, C., Kevers, C., Dommes, J., Pollet, B., Defraigne, J.*, et al.* (2018). The potency of commercial blackcurrant juices to induce relaxation in porcine coronary artery rings is not correlated to their antioxidant capacity but to their anthocyanin content. *Nutrition, 51-52*, 53-59.

Takahashi, M., Ikeda, U., Masuyama, J., Funayama, H., Kano, S., & Shimada, K. (1996). Nitric oxide attenuates adhesion molecule expression in human endothelial cells. *Cytokine, 8*, 817-821.

Tankeu, A.T., Ndip Agbor, V., & Noubiap, J. J. (2017). Calcium supplementation and cardiovascular risk: A rising concern. *Journal of Clinical Hypertension, 19*, 640-646.

Tarbell, J.M. (2010). Shear stress and the endothelial transport barrier. *Cardiovascular Research, 87*, 320-330.

Tham, D.M., Martin-McNulty, B., Wang, Y. X., Wilson, D. W., Vergona, R., Sullivan, M. E., *et al.* (2002). Angiotensin II is associated with activation of NF-kappaB-mediated genes and downregulation of PPARs. *Physiological Genomics, 11*, 21-30.

Therland, K.L., Stubbe, J., Thiesson, H. C., Ottosen, P. D., Walter, S., Sorensen, G. L., *et al.* (2004). Cycloxygenase-2 is expressed in vasculature of normal and ischemic adult human kidney and is colocalized with vascular prostaglandin E2 EP4 receptors. *Journal of the American Society of Nephrology: JASN, 15*, 1189-1198.

Tian,Y., Liimatainen,J., Alanne,A.L., Lindstedt, A., Liu,P., Sinkkonen,J., *et al.* (2017) Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry*, 220, 266-281.

Tigerstedt, R., & Bergman, P. Q. (1898). Niere und Kreislauf1. *Skandinavisches Archiv Für Physiologie, 8*, 223-271.

Tilton, R.G., Chang, K. C., LeJeune, W. S., Stephan, C. C., Brock, T. A., & Williamson, J. R. (1999). Role for nitric oxide in the hyperpermeability and hemodynamic changes induced by intravenous VEGF. *Investigative Ophthalmology & Visual Science, 40*, 689-696.

Tipnis, S.R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., & Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *The Journal of Biological Chemistry, 275*, 33238-33243.

Tjelle, T.E., Holtung, L., Bohn, S. K., Aaby, K., Thoresen, M., Wiik, S. A., *et al.* (2015). Polyphenol-rich juices reduce blood pressure measures in a randomised controlled trial in high normal and hypertensive volunteers. *British Journal of Nutrition, 114*, 1054-1063.

Tonelli, M., Curhan, G., Pfeffer, M., Sacks, F., Thadhani, R., Melamed, M. L., *et al.* (2009). Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. *Circulation, 120*, 1784-1792.

Törrönen, R., Hellström, J., Mattila, P., & Kilpi, K. (2017). Postprandial glycaemic response to berry nectars containing inverted sucrose. *Journal of Nutritional Science, 6, e4*.

Törrönen, R., Kolehmainen, M., Sarkkinen, E., Mykkänen, H., & Niskanen, L. (2012). Postprandial glucose, insulin, and free fatty acid responses to sucrose consumed with blackcurrants and lingonberries in healthy women. *American Journal of Clinical Nutrition, 96*, 527-533.

Törrönen, R., Kolehmainen, M., Sarkkinen, E., Poutanen, K., Mykkänen, H., & Niskanen, L. (2013). Berries reduce postprandial insulin responses to wheat and rye breads in healthy women. *The Journal of Nutrition, 143*, 430-436.

Touyz, R.M., Alves-Lopes, R., Rios, F. J., Camargo, L. L., Anagnostopoulou, A., Arner, A., *et al.* (2018). Vascular smooth muscle contraction in hypertension. *Cardiovascular Research, 114*, 529-539.

Tresserra-Rimbau, A., Rimm, E. B., Medina-Remon, A., Martinez-Gonzalez, M. A., de la Torre, R., Corella, D.*, et al.* (2014). Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutrition, Metabolism, and Cardiovascular Diseases : NMCD, 24*, 639-647.

Tsao, R (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients, 2*, 1231-1246.

Tuomilehto, J., Jousilahti, P., Rastenyte, D., Moltchanov, V., Tanskanen, A., Pietinen, P.*, et al.* (2001). Urinary sodium excretion and cardiovascular mortality in Finland: a prospective study. *Lancet, 357*, 848-851.

Turpeinen, A.M., Jarvenpaa, S., Kautiainen, H., Korpela, R., & Vapaatalo, H. (2013). Antihypertensive effects of bioactive tripeptides-a random effects meta-analysis. *Annals of Medicine, 45*, 51-56.

Valsta, L., Kaartinen, N., Tapanainen, H., Männistö, S., & Sääksjärvi, K. (2018). Nutrition in Finland – The National FinDiet 2017 Survey. *, 12*.

Vane, J.R., Bakhle, Y. S., & Botting, R. M. (1998). Cyclooxygenases 1 and 2. *Annual Review of Pharmacology and Toxicology, 38*, 97-120.

Vanhoutte, P.M. (1989). Endothelium and control of vascular function. State of the Art lecture. *Hypertension, 13*, 658-667.

Vanhoutte, P.M., Shimokawa, H., Feletou, M., & Tang, E. H. C. (2017). Endothelial dysfunction and vascular disease – a 30th anniversary update. *Acta Physiologica, 219*, 22-96.

Vashishtha, V., Barhwal, K., Kumar, A., Hota, S. K., Chaurasia, O. P., & Kumar, B. (2017). Effect of seabuckthorn seed oil in reducing cardiovascular risk factors: A longitudinal controlled trial on hypertensive subjects. *Clinical Nutrition, 36*, 1231-1238.

Virdis, A., Bacca, A., Colucci, R., Duranti, E., Fornai, M., Materazzi, G.*, et al.* (2013). Endothelial dysfunction in small arteries of essential hypertensive patients: role of cyclooxygenase-2 in oxidative stress generation. *Hypertension, 62*, 337-344.

Wallace, T.C., & Giusti, M. M. (2015). Anthocyanins. *Advances in Nutrition, 6*, 620-622.

Wallace, T.C., Slavin, M., & Frankenfeld, C. L. (2016). Systematic review of anthocyanins and markers of cardiovascular disease. *Nutrients, 8*, 10.3390.

Wang, H.D., Pagano, P. J., Du, Y., Cayatte, A. J., Quinn, M. T., Brecher, P.*, et al.* (1998). Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. *Circulation Research, 82*, 810-818.

Wang, Y., Liu, M. J., Yang, H. M., Ma, C. Y., Jia, P. Y., Jia, D. L.*, et al.* (2018). Association between increased serum alkaline phosphatase and the coronary slow flow phenomenon. *BMC Cardiovascular Disorders, 18*, 138-018-0873-6.

Wannamethee, S.G., Sattar, N., Papcosta, O., Lennon, L., & Whincup, P. H. (2013). Alkaline phosphatase, serum phosphate, and incident cardiovascular disease and total mortality in older men. *Arteriosclerosis, Thrombosis, and Vascular Biology, 33*, 1070-1076.

Wehrwein, E.A., & Joyner, M. J. (2013). Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. *Handbook of Clinical Neurology, 117*, 89-102.

Whelton, P.K., Carey, R. M., Aronow, W. S., Casey, D. E.,Jr, Collins, K. J., Himmelfarb, C.*, et al.* (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension, 71*, e13-e115.

Williams, B., Mancia, G., Spiering, W., Agabiti Rosei, E., Azizi, M., Burnier, M.*, et al.* (2018). 2018 Practice Guidelines for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. *Blood Pressure, 27*, 314-340.

Williamson, G., Kay, C.D., & Crozier, A. (2018) The bioavailability, transport, and bioactivity of dietary flavonoids: A review from a historical perspective. C*omprehensive Reviews in Food Science and Food Safety*, 17, 1054-1112.

Wiseman, W., Egan, J. M., Slemmer, J. E., Shaughnessy, K. S., Ballem, K., Gottschall-Pass, K. T.*, et al.* (2011). Feeding blueberry diets inhibits angiotensin II-converting enzyme (ACE) activity in spontaneously hypertensive stroke-prone rats. *Canadian Journal of Physiology and Pharmacology, 89*, 67-71.

Witter, K., Tonar, Z., & Schopper, H. (2017). How many layers has the adventitia? - Structure of the arterial tunica externa revisited. *Anatomia, Histologia, Embryologia, 46*, 110-120.

Wolf, K., Castrop, H., Hartner, A., Goppelt-Strube, M., Hilgers, K. F., & Kurtz, A. (1999). Inhibition of the renin-angiotensin system upregulates cyclooxygenase-2 expression in the macula densa. *Hypertension, 34*, 503-507.

Xie, L., Vance, T., Kim, B., Lee, S. G., Caceres, C., Wang, Y.*, et al.* (2017). Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: a randomized controlled trial. *Nutrition Research, 37*, 67-77.

Xie, W.L., Chipman, J. G., Robertson, D. L., Erikson, R. L., & Simmons, D. L. (1991). Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 2692-2696.

Xu, J.Y., Qin, L. Q., Wang, P. Y., Li, W., & Chang, C. (2008). Effect of milk tripeptides on blood pressure: a meta-analysis of randomized controlled trials. *Nutrition, 24*, 933-940.

Yanagisawa, M, Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y.*, et al.* (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature, 332*, 411-415.

Yang, B, Kalimo, K. O., Mattila, L. M., Kallio, S. E., Katajisto, J, Peltola, O. J*., et al.* (1999). Effects of dietary supplementation with sea buckthorn (*Hippophae rhamnoides*) seed and pulp oils on atopic dermatitis. *The Journal of Nutritional Biochemistry, 10*, 622-630.

Yang, T, & Xu, C. (2017). Physiology and pathophysiology of the intrarenal renin-angiotensin system: an update. *Journal of the American Society of Nephrology : JASN, 28*, 1040-1049.

Zhang, MZ, Yao, B., Cheng, H. F., Wang, S. W., Inagami, T., & Harris, R. C. (2006). Renal cortical cyclooxygenase 2 expression is differentially regulated by angiotensin II AT(1) and AT(2) receptors. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 16045-16050.

Zhang, Z, Zhou, Q., Huangfu, G., Wu, Y., & Zhang, J. (2019). Anthocyanin extracts of lingonberry (Vaccinium vitis-idaea L.) attenuate serum lipids and cholesterol metabolism in HCD-induced hypercholesterolaemic male mice. *International Journal of Food Science and Technology, 54*, 1576-1587.

Zheng, W, & Wang, S. Y. (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry, 51*, 502-509.

Zhu, Y, Sun, J., Lu, W., Wang, X., Wang, X., Han, Z*., et al.* (2017). Effects of blueberry supplementation on blood pressure: a systematic review and meta-analysis of randomized clinical trials. *Journal of Human Hypertension, 31*, 165-171.